Final Report: Practical Soil Test Methods for Predicting Net N Mineralization

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Executive Summary

Inappropriate application rates and timing of fertilizer and manure N have been implicated in high groundwater nitrate content in several regions of the state. In a few cases, N from agricultural sources has been blamed for low dissolved oxygen content in surface waters. For example, hypoxia symptoms in the Gulf of Mexico and in the shipping canal of Stockton, California are considered to be caused from over N fertilization in upstream agricultural lands. So far, nutrient management professionals and university researchers have focused on soil testing for nitrate and matching N application rates and timing to actual crop uptake. One of the main technical reasons for the inability to determine application rates and timing of N fertilizers is that no good soil test method exists to predict soil N availability throughout the growing season. The barrier to developing a method to predict N availability is in the uncertainty in the amount of available N made available during a growing season from mineralization of recent crop residues, soil organic matter, and organic amendments. Across soil types and cropping practices, the quantity of N mineralized from soil annually ranges from very low (perhaps <20% of plant N requirement) to well above the plant N requirement. Until this uncertainty is addressed, the possibility of over applying fertilizer N remains and the environmental consequences of excess fertilization will remain an issue. Numerous soil analytical methods for mineralizable N have been published, and many have been shown to be effective under experimental and certain field conditions. Generally, these methods either require too much time to conduct (half year incubation in the laboratory) or are otherwise not suited for commercial laboratory usage; or they are effective only within a narrow set of soil and cropping conditions. In a recent breakthrough, researchers at the University of Illinois (Khan et al. 2001) have developed a technique for measuring one soil organic N fraction – amino sugars – that they have shown to be highly predictive of N fertilizer responsiveness. The intent of this project was to evaluate the soil amino sugar test as a predictor of available soil N.

We successfully modified and streamlined the soil amino sugar test for commercial laboratories eliminating the need for bulky analytical glassware and minimizing hazardous waste production from the original method. The modified soil amino sugar test method was compared to numerous other soil tests including preplant soil nitrate, presidedress soil nitrate, anaerobic incubation, aerobic incubation, hot potassium chloride, soil carbon dioxide evolution, crop N uptake, total soil N and total soil carbon. The modified soil amino sugar test was comparable to total soil N predicting approximately 70% of the observed variation in crop N uptake. Two independent commercial laboratories tested the modified soil amino sugar test and produced comparable results to each other and were within 5% of the values determined by our analysis. Our soil amino sugar test results for California agriculture soils indicate that a value of 100 ppm amino sugars predicted fertilizer N response in contrast to 220 ppm for Midwestern studies. This discrepancy is likely related to California agricultural soils containing less than half of the total soil N and C than Midwestern soils. We shared our results with various grower and technical working groups to satisfy the outreach component of the project. The product of this research will enable soil test labs and agriculture professionals to provide additional information to growers determine whether crops would be responsive to N fertilizer applications and to fine tune application rates. The modified soil amino sugar test was better than most soil N tests at predicting crop N uptake satisfactorily, however, it was no better in its predictive value than the total soil N determination.

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Project Justification

Mismanagement of N in fertilizers and livestock manure has led to environmental problems: Elevation of nitrate in groundwater used for municipal and rural domestic supply above the acceptable level, and increase of hypoxia in the Gulf of Mexico and possibly in the Stockton deep water shipping channel. Adjustments to rate and timing of N to crops are widely accepted as the most effective method for reducing water pollution. Highly effective methods for doing this are not so apparent. A commonly advocated farm management practice – soil testing for nitrate – is not always effective, and in any case has not been widely adopted in California or anywhere else. The obvious weakness of soil testing for nitrate is that it usually does not provide an estimate of N mineralized from the organic matter during the crop season.

Most of the soil N (~95%) is in the organic form. Many studies have shown that commonly in cultivated soils, two to five percent of organic N in soils is mineralized annually. The range of uncertainty in this percentage is especially wide where climate, soils, use of livestock manure, cover crops and crop rotation are highly variable or diverse, as is true for California's agriculture.

In recent years, soil nitrate testing procedures – for example, the PSNT (pre-sidedress nitrate testing) and PPNT (preplant nitrate testing) have been advocated and to some extent adopted in the Midwest and Northeast US (Bundy and Andraski. 1995). Magdoff et al. (1994), improved the PPNT by developing the presidedress nitrate test (PSNT). The PSNT samples available soil N within days of sidedressing fertilizer. In some instances, soil nitrate testing may be the best option for California farmers to reduce over-fertilization. However in several situations common in California, soil nitrate testing is quite ineffective, in particular, where root systems are shallow, due to claypan or hardpan conditions and/or where the leaching fraction is unavoidably high (coarse-textured soils irrigated by furrow or border check), soil nitrate content does not reflect the soil N mineralization potential.

The limitation of testing for soil NO_3^- is related to the dynamic nature of the soil N cycle. The temporal nature of the available soil N pool negates the predictive capacity of the tests based solely on the amount soil NO_3^-. Therefore, in practice the PPNT and PSNT have only been marginally acceptable in assessing fertilizer N application rates. For example, Krusekopt et al. (2002) often found little correlation of PPNT to tomato yields. Similar observations have been found in cotton (B. A. Roberts, personnel observation). Even though these results seem not to be promising, soil testing for available N is still considered the best option to determine sites where N fertilization will produce a yield response.

Recent evidence points to amino sugars as being useful to determine soils that are responsive to N fertilization (Khan et al. 2001). Amino sugars account for up to 25 to 45% of the total soil N. Amino sugars in soil N are primarily derived from microbial and faunal sources. The size of the amino sugars pool will depend on the activity of the microbial biomass. The production (microbial growth) and subsequent deposition (microbial turnover) of amino sugars in soil is directly related to available soil N. The higher the available soil N pool, the more likely the amino sugar pool will also be high. The advantage of examining the amino sugar pool in soil is

that it represents an index describing a fraction of soil N that contributes significantly to soil N availability. In addition, the timing of sampling is not as critical as for the PPNT or PSNT methods since the amino sugar fraction represents the activity of the microbial biomass, which is more stable than the dynamic nature of soil nitrate.

Though the amino sugar technique has shown great promise in higher organic matter soils of the Midwest, its application in California needs assessing before it can be adopted. Testing is needed on low-organic matter, irrigated soils found in California. The challenge in testing and implementing the amino sugar method will be to determine soil characteristics, such as non-nitrogen nutrient levels and soil organic matter characteristics, which may affect the interpretation of the assay.

Background

Nitrogen in the environment

Nitrogen (N) occupies a unique position among the soil-derived elements essential for plant growth because of its complex biogeochemistry and the rather large amounts required by most agricultural crops in comparison to other elements. In plants, N is a constituent of chlorophyll, all proteins, including the enzymes, and many other compounds. Although many productive mineral soils contain several thousand kilograms of N per hectare, about 90% of the soil N is unavailable in the form of organic matter, and most of the remainder exists as fixed ammonium in clays (Foth and Ellis, 1997). Only a small fraction of the N in soils, generally less than 0.1%, exists in plant-available mineral compounds, such as nitrate (NO_3^-) and exchangeable ammonium (NH_4^+), at any one time, and no more than 1-2% of the total soil N will be available to plants during a growing season (Stevenson and Cole, 1999). The large need of plants for N and the limited ability of soils to supply it cause this nutrient to be the most limiting for crop production on a global basis (Foth and Ellis, 1997). In intensive agricultural systems, N is therefore applied in large amounts to promote productivity.

While insufficient application of N can have serious economic consequences for the farmer, excessive fertilization increases the risk of environmental pollution, because the N cycle in soils is not a closed system (Figure 1). Gains in soil N in agricultural systems occur mainly by application of fertilizer and normally in much smaller quantities by microbial fixation of molecular N2 and by atmospheric precipitation. Losses occur through leaching, denitrification, volatilization, erosion, and in agricultural systems through crop removal. The leaching of nitrate in agriculture has received considerable attention in recent years because of possible pollution of groundwater. In California, groundwater supplies more than 40% of the water demand (Zhang et al., 1998). One well-known potential health threat is the relationship between high nitrate levels in drinking water and a rare infant disease called methemoglobinemia, or blue-baby syndrome. This disease occurs because in the stomachs of young babies, nitrate can convert to nitrite which interferes with the blood's oxygen-carrying capacity. Cancer and birth defects are other concerns related to nitrate; however no firm link has been established (Franco and Cady, 1997). According to a report by the State Water Resource Control Board (Anton et al., 1988), about 10% percent of the water samples taken from Californian supply wells showed nitrate concentrations exceeding the state's maximum contaminant level of 45 mg NO_3^-/L, which is

equal to 10 mg NO_3^--N/L. Agricultural activities have been suspected to be one of the largest sources of nitrate in Californian groundwater (Anton et al., 1988).

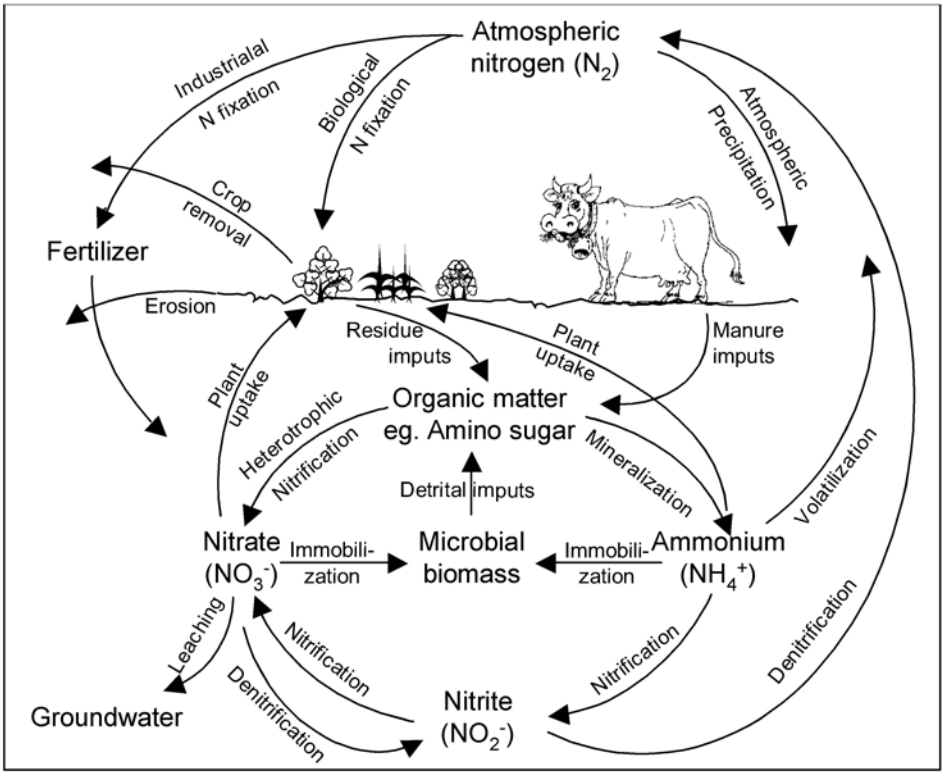


Figure 1. The N cycle in the soil. The many steps in the N cycle can lead to losses in both gaseous and dissolved form.

Nitrogen fertilization and fertilization recommendations

Even under ideal conditions, no more than two-thirds of the N added as fertilizer is generally removed by crops or recovered in the soil at the end of the growing season; losses as much as one-half of applied fertilizer N are not uncommon (Stevenson and Cole, 1999; Giller et al., 2004; Balasubramanian et al., 2004). As an example, Zhang et al. (1998) found that the N not used by field crops accounted for 13 to 53% of the fertilizer N added to crops in Tulare county (Table 1). The low efficiency of N fertilization is due to the highly dynamic properties of the N cycle in soils. Transformations from one N form into another are mainly mediated by soil microorganisms which are affected by a number of factors, including temperature, water content, oxygen availability, pH, supply of nutrients, soil texture, as well as organic matter content and quality (Appel and Mengel, 1990). The ability of a soil to provide plant-available N through mineralization varies considerably and is therefore difficult to estimate.

Table 1. Annual nitrogen and yield relationship among field crops grown in Tulare county during the 1980s (Zhang et al., 1998).

Commodity	Yield (kg/ha)	N applied (kg/ha)	N removed (kg/ha)	Excess N after removal by crop (kg/ha)	%
Barley	4 500	97.6	85.0	12.6	13
Wheat	4 500	166.7	79.0	87.7	53
Grains, other	4 000	112.0	73.3	38.7	35
Cotton seed	1 900	126.2	67.0	59.2	47
Sugar beets	56 000	145.6	73.0	72.6	50
Corn	10 000	215.6	190.0	25.6	12

Nitrogen fertilization recommendations that take into account a soil's ability to mineralize N during the growing season are highly desirable as they would increase the efficiency of added N, be economically beneficial for the farmer, and reduce groundwater pollution. Many methods to assess the N mineralization capacity have been proposed since the early 1900s (Bundy and Meisinger, 1994). These approaches can be divided into three main groups: Soil tests, plant tests, and models, which combine several factors. Soil tests assess the N mineralization potential with biological or chemical procedures. Plant tests measure plant properties directly related to the N status of the plants, such as yield, tissue N concentration, and N uptake. Studies have been carried out either in the field or in the greenhouse. Models integrate soil, plant and climatic factors and their interactions and may include soil or plant tests as inputs.

Characteristics of an ideal test

The ideal indicator is closely related to the N status of the soil-crop system and must be able to detect or predict both N deficiency and N excess. Ideally, indicator values must not be affected by any factors other than the N status of the soil-crop system, which means that a certain N status must yield the same indicator value within sites and across sites and years (Schröder et al., 2000).

The time for sampling or testing should not fall into a period of major farm activities, and the costs of a test have to be lower than the potential savings due to reduced application of fertilizer. This means that a single analysis has to be carried out in a short time and that the costs for equipment and reagents per test have to be low. The analysis has to be carried out in a short time because indicators directed at N use in the current season must inform growers rapidly to allow for a timely supplementation of N.

Soil tests

Field Soil Tests

Many field methods have been used to quantify N mineralization such as buried bag, ion exchange resin methods, and soil nitrate tests (Rice and Havlin, 1994). In agriculture, only the soil nitrate test has found widespread application.

Soil nitrate test

Samples for the soil nitrate test are usually taken before planting (Pre-plant Nitrate Test, PPNT), or before sidedressing nitrogen fertilizer (Pre-sidedress Nitrate Test, PSNT). The PSNT is the most widely used nitrogen test. It has been developed for corn in the humid regions of the Midwest and Northeast of the United States. The PSNT has succeeded in corn because corn is a warm-season crop, which allows ample time for N mineralization in spring before the crop begins its period of rapid N uptake (Bundy and Meisinger, 1994). The test measures the amount of nitrate in the soil just prior to the time that it would be sidedressed with N (Magdoff et al., 1984 and Magdoff, 1991). Soil samples are generally taken from the upper 30 cm soil layer. Although the test takes into account some of the year to year variability, the PSNT often cannot accurately predict the amount of nitrogen fertilizer the farmer has to add. The nitrogen availability may be underestimated when intensive precipitation just before sampling leaches nitrate below the sampling depth or when cool and wet weather causes low rates of N mineralization before the sampling and the weather pattern changes afterwards. On the other hand, N availability may be lower than predicted when intensive rainfalls after sampling leach significant quantities of nitrate below the root zone (Magdoff, 1991). For this reason, Andraski and Bundy (2002) found that the accuracy of the PSNT depended on climatic and soil factors. Despite these limitations, N rate recommendations based on the PSNT yielded higher economic returns than N rate recommendations unadjusted for recent N contributions from manure and legumes (Andraski and Bundy, 2002). Furthermore, basing the N rate recommendation on the PSNT reduced potential N losses to the environment. On the other hand, Fox et al. (1989) found that the PSNT is good for predicting N fertilizer response but not good for making accurate N fertilizer rate predictions, and Hong et al. (1990) found only a moderate correlation between the PSNT and the N-supplying capacity of the soil.

Biological laboratory methods

In biological tests, a soil sample is incubated under temperature and moisture conditions conductive to N mineralization. After a given time period, the total mineral N produced is measured. Many variations of this basic procedure exist, but in general, biological methods are divided into two major groups: aerobic and anaerobic incubations.

Aerobic incubation

Many procedures have been proposed for aerobic incubations. They differ in their incubation time, temperature and whether mineral N is determined destructively or by repeated leaching of a sample. Correlations between biological procedures and greenhouse results are generally good, but when testing progresses to field conditions, the correlations are usually considerably lower or non existent. The following reasons may be responsible:

1. Measurements of N mineralized from disturbed soil samples often overestimate field N availability due to stimulation of mineralization by drying, crushing and sieving the soil (Bundy and Meisinger, 1994; Cabrera and Kissel, 1988). Bremner (1965) found that short-term aerobic incubations are dependent on methods used in pretreating the soil before incubation. Even with the use of rigorously standardized methods, results of short-term incubations do not necessarily reflect the potential, long-term N supplying capacities of soils (Stanford and Smith, 1972). Nitrogen mineralized during incubation of undisturbed soil cores can provide a more reliable assessment of N availability. However, the relatively large number of undisturbed soil cores are needed due to soil spatial variability which often makes this approach impractical for field-scale N mineralization predictions by soil test labs (Cabrera and Kissel, 1988).

2. Nitrogen mineralized in short periods under aerobic conditions may be influenced by the N derived from decomposition of recently incorporated residues and microbial tissues (Stanford et al., 1975). On the other hand, N immobilization due to recently incorporated residues with a high C/N ratio may lead to an under-prediction of the N mineralization potential of the soil (Chichester et al., 1975).

Long-term incubations estimate the mineralization potential of soils better, but this approach is, expensive and time-consuming (Stanford, 1982). The most serious drawback of this approach is that the long incubation time does not allow for in season estimation of N fertilizer application rates.

Despite these limitations, it is generally recognized that aerobic incubations that produce nitrate-N and ammonium-N provide a sound relative measure of the N mineralization potential of a soil. This is because the soil organic N is released by the same biological processes active under field conditions.

As the C and N cycles in soil are closely linked, CO_2 evolution has been found to correlate with N mineralization (Franzluebbers, 2000). Aerobic incubations allow for the determination of both mineral N and CO_2, which may reveal interesting aspects of the decomposition process.

Anaerobic incubation

Incubation of soils under anaerobic (waterlogged) conditions was initially proposed by Waring and Bremner (1964), who found a close relationship between the amount of ammonium-N produced under waterlogged conditions and the amount of mineral nitrogen produced by incubation under aerobic conditions. In general, an incubation time of 7 days at 40° C sufficient to predict field N availability. Compared to the aerobic incubation method, the anaerobic incubation has the following advantages (Waring and Bremner, 1964; Stanford, 1982):

- It is simple, rapid, precise, and readily applicable to disturbed air-dried or field-moist soils.
- It does not require the use of amendments or preliminary analyses to determine the amount of water required for incubation and constant water content doesn't have to be maintained as in aerobic incubations.

- The containers do not need aeration.
- Only ammonium is produced making analysis similar.
- Because little or no nitrate is produced, the results are not affected by differences in denitrification.
- The apparatus and reagent requirements are minimal.
- The incubation time is short.

Although reviews have cited numerous reports of satisfactory relationships between results from anaerobic incubation method and crop N uptake (Stanfod, 1982; Meisinger, 1984), several studies have found poor correlations between ammonium-N production under waterlogged conditions and field measurements of N availability (Fox and Piekielek, 1984). These differences may not be contradictions, but instead reflect differences in N transformations measured by the two methods. Specifically, field measurements represent the net effect of N mineralization and N immobilization under aerobic conditions, while the waterlogged incubation likely measures N mineralization from aerobic soil organisms killed by the anaerobic test conditions (Bundy and Meisinger, 1994). Studies determining the correlation between anaerobic net N mineralization and fumigation-incubation microbial biomass N and C have obtained inconsistent results. Sometimes the correlation was very strong, consistent with the hypothesis that both techniques are measuring N mineralized from the microbial biomass N pool, however in many cases there appeared to be no relationship (Drinkwater et al., 1996).

Chemical laboratory methods

Chemical methods for assessing N availability are appealing because they offer a simple and rapid approach to estimate crop N fertilizer needs and a convenient assessment of relative differences among various experimental treatments in research projects (Bundy and Meisinger, 1994).

The goal of chemical methods is to identify all or a part of the active N pool which is closely related to the N supplying capacity of the soil. Proposed extracting agents vary greatly in intensity, ranging from strong acids/bases to neutral salts or water. The general conclusion from research and reviews is that intensive extracts remove large amounts of soil N and are usually related to total N; while extracts of mild intensity remove an active soil N component (Stanford, 1982).

Hot KCl

In this method, first proposed by Gianello and Bremner (1986a), a soil sample is heated with 2M potassium chloride (KCl) for four hours in a stoppered tube placed in a block digester or a water bath maintained at 100°C, and the ammonium-N released is analyzed. Tests using 50 organic N compounds, showed that only few organic compounds, mainly amino acids, amino sugars, and amines, are released with this method (Gianello and Bremner, 1986a).

An advantage of the hot KCl method is that the result is not affected by air drying and air dry storage (Gianello and Bremner, 1986a). The method is rapid and simple and does not require hazardous chemicals.

The length of heating and the temperature have significant effect on the N released (Gianello and Bremner, 1986a). A strict protocol is therefore required to obtain reproducible results. This may be more difficult when using a water bath to heat the samples, as the water cools down when the samples are placed into the bath. Another important point is that the tubes are well sealed, as otherwise ammonia losses (NH_3) may affect the result (Gianello and Bremner, 1986a).

Phosphate borate buffer

In this method, a soil sample is steam distilled with pH 11.2 phosphate-borate buffer solution for 8 minutes and the ammonia-N produced is measured (Gianello and Bremner, 1988). The method is simple and precise and the results are not significantly affected by air-drying and air dry storage of the soil samples before analysis (Gianello and Bremner, 1988).

Direct diffusion method (Illinois Soil Test)

An interesting alternative soil test has been proposed by Khan et al. (2000 and 2001). In this method, soil samples are treated in closed Mason jars either with KCl and magnesium oxide (MgO) to convert ammonium-N to ammonia or with sodium hydroxide (NaOH) to convert ammonium-N and amino sugar-N to ammonia. The jar is placed on an electric griddle maintained at 48 to 50° C for five hours. The ammonia liberated is collected in boric acid (H_3BO_3) indicator solution in a Petri dish suspended from the Mason jar lid and determined quantitatively by acidimetric titration. The difference between the two analyses represents mainly the amino sugar content of the soil. An insignificant amount of N from amino acids may also contribute to the N released during the assay (Mulvaney and Khan, 2001).

A limitation of the alkaline decomposition method is that amino sugar-N is calculated from the difference between the amount of ammonium recovered by two diffusion procedures. Accordingly, the method is unsuitable for analysis of samples containing low concentrations of amino sugar in the presence of relatively high concentrations of ammonium.

UV absorbance of $NaHCO_3$

In this method, a soil sample is shaken with 0.01 sodium bicarbonate ($NaHCO_3$) for 15 minutes. The suspension is filtered and the UV-absorbance of the extract is measured at either 260 nm or 200 nm with a UV-spectrophotometer (Fox and Piekielek, 1978b). The UV absorbance at 260 nm is an estimate of the organic matter content of the $NaHCO_3$ extract (Fox and Piekielek, 1978b), and the absorbance at 200 nm is a function of both the nitrate and organic matter content of the extract (Norman et al., 1985). While Fox and Piekielek (1987b) found a good correlation between the UV absorbance at 260 nm and the N supplying capacity of the soil, Hong et al. (1990) were more successful in relating the UV absorbance at 200 nm to the soil N availability. This method could be interesting for routine prediction of the soil N-supplying capacity because the procedure is simple and large numbers of samples can be processed in a short time.

Assessing nitrogen mineralization potential with chemical methods

In general, the hot KCl and phosphate-borate buffer methods seem to be well correlated with the N supplying capacity of a soil. Several studies obtained close correlations between biological methods and hot KCl and phosphate-borate distillation method (Gianello and Bremner, 1986a; Gianello and Bremner, 1986b; Jalil et al., 1996). The good correlation may be due to the fact that the soil N fraction these two methods measure is not only similarly available under the conditions of the treatment but it is also composed of a relatively small number of compounds, namely amino sugar and certain amino acids (Gianello and Bremner, 1986a). These compounds may also be similarly accessible to microbial degradation. However, when these and other chemical indices are compared with crop growth in the field, the relationship is often weak. Hong et al. (1990), for example, found a weak correlation between the hot KCl and phosphate-borate buffer method and the N-supplying capacity of the soil in a field study. The correlation was markedly improved when the soil nitrate concentration was added to the N measured by these two methods. This approach makes sense, as the plants can use both the nitrate already present at sampling time and the nitrogen mineralized during the cropping season.

The direct diffusion method has not yet been validated with independent samples. As it measures a similar group of organic N-compounds as the hot KCl and the phosphate-borate buffer method, it has a potential of being at least as well related to N mineralization as these two methods.

Plant tests

The most widely used plant tests measure plant properties directly related to the N status of the plants, such as N uptake and tissue N concentration, or they measure properties closely related to N uptake, such as leaf greenness.

Crop N uptake

Determining the N uptake of a field crop is the most satisfactory method of estimating the soil N supply, because it integrates the factors of crop growth and soil N dynamics under field conditions. Crop N uptake has been generally accepted as the standard by which other more empirical methods are evaluated (Meisinger, 1984), but it relies on the assumption that no N is added to or removed from the system, so that N mineralization and residual mineral N will be the major N sources on plots without N fertilizer application. This assumption may not always be valid. For example, in the early stages of crop growth, N mineralization often exceeds crop N uptake. Heavy rainfall or irrigation may lead to N losses by leaching of nitrate out of the rootzone. Another disadvantage of N uptake determination is the large time and labor requirements. Since N uptake integrates field conditions over time, it is also quite site specific, and shows significant year by site interactions (Meisinger, 1984; Rice and Havlin, 1994). The results from one site are not easily transferred to other sites with different environmental conditions. Furthermore, the results are not available until after harvest, and cannot be used to adjust nitrogen fertilization in the same season. Nevertheless, field measurements of N uptake are essential for calibrating chemical and biological estimates of N mineralization for use in N recommendation models (Rice and Havlin, 1994).

Greenhouse procedures have the advantage that many environmental factors can be controlled, but they have limited application to field conditions. Measuring N uptake in the greenhouse still serves a useful purpose in the preliminary screening of more empirical chemical procedures.

An alternative to measuring N uptake is to determine crop response to N fertilization by comparing the yield and N uptake of plants grown on zero N plots with fertilized plants in the same field. This procedure minimizes the effect of factors such as climate, management practices, and variety, but requires twice as much labor for crop harvest and analysis. Furthermore, it relies on the assumption that the growth of the fertilized plants was not limited by N availability.

Plant tissue nitrate

A positive relationship can be found between the availability of soil mineral nitrogen and the nitrate concentration of a crop. This relationship is not linear, however, and it is difficult to translate observations on nitrate concentration into quantitative statements on N deficiency or excess (Schröder et al., 2000). The nitrate concentration does not uniquely reflect the crop's N status, as the nitrate concentration drops during plant development (Binford et al., 1990; Geyer and Marschner, 1990) and differs between plant organs (Geyer and Marschner, 1990). A test, therefore, has to be carried out at a fixed growth stage analyzing a clearly defined plant part. Preferably, the test is to be carried out at an early stage in order to permit adjustment of the N fertilization for sidedress.

Fox et al. (1989) compared the stalk nitrate concentration of corn plants in the five to six leaf stage with the N supplying capacity of the soil. In their study, the basal 10 cm of corn stalks were dried, ground and analyzed for nitrate concentration. The resulting correlation between stalk nitrate and N supplying capacity of the soil was weak, especially for soils that did not receive animal manure the previous two years. In these fields, solar radiation and soil moisture affected stalk nitrate concentration. Fox et al. (1989) concluded that the test was not accurate enough to be used over a range of climatic conditions. Geyer and Marschner (1990) found that measuring the nitrate concentration of the blades of the two oldest, photosynthetically active leaves is a very useful method for evaluation of the N nutritional status of maize when taking into account both plant age and N mineralization potential of the soil.

Plant total N

Experiments on the response of maize to N application have shown positive relationships between yield and either the total N concentration of young, 15 to 30 cm tall maize plants (Binford et al., 1992), or the total N concentration of the ear leaf around silking (Cerrrato and Blackmer, 1991). However, both studies found a weak correlation between the total N concentration and amounts of available N in the soil. The N status of corn was an unsuitable indicator for the detection of excess N. Moreover, due to the considerable variation among sites and years, a critical N level, beyond which response to N is unlikely, is difficult to determine (Cerrato and Blackmer, 1991; Binford et al., 1992).

Crop color

Recently, the development of indicators has focused on the use of color. These indicators either refer to the color of individual leaves (leaf greenness) or to the color of the entire crop (field greenness). Most of the time, field greenness is assessed with hand-held portable reflectometers or with reflectometers from airplanes, whereas leaf greenness is assessed with a hand-held chlorophyll meter, which measures the absorbance of the leaf in two wavelength regions (Konica Minolta, 2003). Leaf greenness is closely related to chlorophyll and N concentration in the leaf (Dwyer et al., 1991; Schepers et al., 1992). However, factors such as crop growth stage, hybrid, cultural practices and water stress affect the reading (Piekielek and Fox, 1992; Waskom et al., 1996; Schepers et al., 1996). Like nitrate plant tests, greenness tests are unable to detect excessive N availability (Schröder et al., 2000). Nitrogen deficiency, however, is immediately reflected in a low chlorophyll concentration, making this test suitable to detect N deficient conditions.
The advantages of measuring leaf or field greenness are that the results are immediately available and that the plants are not damaged. A large area can be sampled easily and quickly and the results can be used to assess spatial variability in a field. The value of the results can be increased by comparing the reading with a well fertilized reference strip in the same field (Waskom et al., 1996; Piekielek and Fox, 1992). This method allows for detection of N deficiencies and in-season correction of fertilizer N application. Varvel et al. (1997) found that the maximum yield was attained in fields where the reading did not drop below 95% of the reading from the reference plot.

Plant tests versus soil tests

There are arguments in favor of both soil and crop analysis. From an economical point of view, it is the N status of the crop rather than the N status of the soil that is relevant to a producer (Schröder et al., 2000). Crops integrate factors such as the presence and availability of soil mineral N, weather, and agronomic management (Binford et al., 1992). Strong points in favor of crop-related indicators are the relatively low labor requirements and expenses. In addition, the lag time between sampling and prescription is generally smaller for crop than soil related indicators. This can be essential for a timely correction of the N supply.

Questions have been raised whether a young crop properly predicts the N status over the entire season. Indicators based on crop analyses are unable to fully reflect the N mineralization potential of a site when soil water deficits have temporarily excluded parts of the soil profile from root growth (Schröder et al., 2000). Another limitation of most plant tests is their qualitative nature. They indicate whether the crop under study is likely to respond to sidedress N, but they do not quantify how much N must be added (Schröder et al., 2000).
Furthermore, crop analyses are not suited to detect excess N, especially because the uptake of N and its subsequent conversion into structural compounds becomes saturated at some upper limit (Geyer and Marschner, 1990; Magdoff, 1991; Roth et al., 1992; Bundy and Andraski, 1993; Dwyer et al., 1991).

Modeling N mineralization

A simple model, based on aerobic incubation, has been proposed by Stanford and Smith (1972). Periodic measurement of the mineralized N allows for description of the relationship between cumulative N mineralization (Nt) and time (t) of incubation. Assuming first-order kinetics, Stanford and Smith (1972) proposed an exponential equation to estimate the N mineralization potential (N_0) of a soil:

$$Nt = N_0 (1\text{-}e\text{-}kt), \qquad \text{eq. 1.1}$$

where k is the specific rate of mineralization. Several other approaches have been used to mathematically infer N_0 from the cumulative amount of N mineralized by a soil at various times (Nt). Another widely used equation is the double exponential model, first proposed by Molina et al. (1980):

$$Nt = N_0 S (1\text{-}e\text{-}ht) + N_0 (1\text{-}S) (1\text{-}e\text{-}kt), \qquad \text{eq. 1.2}$$

where S and 1-S represent the labile and recalcitrant organic N fractions decomposing at specific rates h and k, respectively. The double exponential equation allows for partitioning of the N_0 value. In general, the double exponential model has been found to more closely fit N mineralization data obtained from aerobic incubation studies (Molina et al., 1980; Deans et al., 1984, Dou et al., 1996). This can be expected, since soil organic matter has heterogeneous components with varying degrees of degradability. Any model having multiple pools should be more accurate than a single pool model in describing soil N mineralization (Dou et al., 1996).

N_0 provides a common basis for evaluating various chemical and biological availability indices under a broad range of soil conditions and for making quantitative estimates of N mineralization in the field. However, attempts to relate the biologically determined pools to soil properties have not been very successful. This may be due to the influence of the incubation time on N_0 and k (Cabrera and Kissel, 1988), and to the fact that k is not a constant, as assumed in the exponential models. Sierra (1990) found that the mineralization rate is not a constant but a variable depending on the availability of the mineralizable N pool. This is in agreement with the common knowledge that as the N mineralization proceeds, the mineralizable N pool becomes less available and the mineralization rate slows.

As mentioned before, incubations carried out at constant and favorable conditions do not reflect the constantly changing conditions in the field. A more dynamic approach is therefore needed. With the widespread use of increasingly powerful computers, models became more and more elaborate. These models are dynamic and integrate climatic, soil and plant factors. Although current models include functions for the important soil and plant processes, their use is still relatively limited. This is due to the fact that it is difficult to calibrate the models, as some of the presumed functional pools cannot be quantified by physical, chemical and biological techniques. Consequently, the models have to be calibrated by adjusting the rate coefficients and pool sizes to fit the measured data (Elliot et al., 1996). Therefore, the performance of a model depends on site-specific calibration. In the future, a major challenge will be to free simulations from site-specific calibration and to devise experimental methods for providing initial values to run a model (Benbi and Richter, 2002).

This brief review summarized only the most widely used and most promising new methods. The soil tests that seem most suitable to assess N mineralization for fertilization recommendations will be evaluated in a field study (Chapter 7). Extensive reviews of the methods proposed over decades of research can be found in Stanford (1982), or Bundy and Meisinger (1994).

Objectives

1. Develop one or more commercially practical soil analytical procedures for measuring available soil N during the growing season of low-organic matter-irrigated soils in various rotations and crops, and on both manured and non-manured sites. The primary candidates for analytical methods are the recently developed soil amino sugar method (Kahn et al. 2001, University of Illinois) and modifications thereof.

2. Collaborate with commercial soil test labs for method adoption and quality control.

3. Conduct an outreach program to train crop advising and analytical laboratory staff, growers and other interested persons in the use of the soil N mineralization test.

Summary of Task Completion

Task 1, year 1. Identify responsive and non-responsive sites to N fertilization and collect soils.

This task is completed. We will continue to expand soil sampling to encompass more soil and cropping system types.

Task 2, year 1. Characterize these soils physically and chemically.

This task was completed in the fall of 2004.

Task 3, year 1. Determine potentially mineralizable N using established methods and test amino acid sugar assay. The methods include:

a. NO_3^- release during aerobic incubation.
b. CO_2 evolution.
c. Direct diffusion method for amino sugar N.
d. Hot KCl extraction
e. Anaerobic incubation

This task was completed in the fall of 2004.

Task 4, year 1. Characterize the fertilizer N responsiveness of sites.

The comparison of fertilizer treatments is required to verify the usefulness of the amino sugar method. In addition, in comparing the amino sugar method with the other methods we hope to the results will help us interpret weak correlation or reasons to explain or improve the technique. This task was completed in the fall of 2004.

Task 5, year 1. Modify amino sugar method to increase production efficiency for use in soil test labs.

The scaling down the methodology to a micro setup will facilitate sample throughput and reduce space requirements. This task was completed in the spring of 2005. After conferring with soil test labs, some adjustments are required to make the method more user friendly for soil test labs. Completed spring 2006.

Task 6, year 2. Identify additional responsive and non-responsive sites to N fertilization and collect soils.

The identification of additional sites was completed to encompass a greater range of soils as well as soil factors identified from the interpretation of the results from year one. Soil sampling was completed 2 to 3 weeks after planting in the spring of 2005.

Task 7, year 2. Characterize additional soils collected in year two physically and chemically.

The basic soil analyses will provide data to interpret variation in the results as a consequence of differing soil properties and cropping histories. This task was completed fall of 2005.

Task 8, year 2. Determine potentially mineralizable N using established methods and test amino sugar assay on soils collected in the second year.

See details on task products in **Task 3**. This task was completed in 2005. The results are included in this report.

Task 9, year 2. Characterize the fertilizer N responsiveness of sites.

The comparison of fertilizer treatments is required to verify the usefulness of the amino sugar method. In addition, in comparing the amino sugar method with the other methods we hope to the results will help us interpret weak correlation or reasons to explain or improve the technique. This task was completed in 2005.

Task 10, year 2. Facilitate transfer of amino sugar method to commercial soil test lab for comparison of results to UC Davis and to assess issues about industry adoption.

We hope to produce a new method to assess the fertilizer N responsiveness of soil and sites with specific cropping histories. If successful the method would be used to identify sites that are not responsive to N fertilization. This task includes collaborating with soil test labs to assist in the adoption of the amino We expect this task to be completed by winter 2006.

Task 11, year 3. Continue soil testing and finalize standard operating procedure for amino sugar method and develop value and sliding scale approach for the interpretation of the assay.

Verification of results, standard operating procedure and interpretive guide are the products of this task. Completed Spring 2006.

Task 12, year 3. Develop and conduct an outreach program.

This task is on-going and will be completed in winter 2006-07.

Work plan and Methods

We investigated the amino sugar method and our own modified soil amino sugar test (SAST) along with several other methods. The comparison with other methods was required to determine the robustness and reproducibility of the SAST. The comparisons aided us in interpreting the results of the SAST and provided data on the success of the procedures on different soil types. We concentrated our efforts on corn since this is the crop that the original amino sugar method is based. Most importantly the methods examine similar soil N fractions and provide for the opportunity to determine which method more accurately describes the labile soil N fraction most likely contributing to the mineralizable soil N pool. The work done on this project was defined by deliverable tasks shown below.

The following discussion is arranged by task.

Task 1, year 1. Identify responsive and non-responsive sites to N fertilization and collect soils.

and

Task 2, year 1. Characterize these soils physically and chemically.

The following describes methods for soil sampling and methods to characterize soil properties during the first and second year of the project.

Soil sampling and preparation

Soil samples were taken in the field with a 1.2 cm auger. At least five cores were taken per plot and stored in an ice chest. In the lab, the samples were dried in an oven at 40° C. The dry samples were ground to pass a 2 mm sieve and stored in plastic bags.

Gravimetric soil moisture content

Soil samples were placed into tin pans, weighed and dried in an oven for 24 hours at 105° C and weighed again (Gardner, 1986). The gravimetric soil moisture content (ω) was calculated as follows:

$$\omega = \frac{\text{weight of moist soil - weight of oven dry soil}}{\text{weight of oven dry soil}} \qquad \text{eq. 2.1}$$

In this thesis, all concentrations of soil constituents are reported on an oven dry (OD) basis.

Water holding capacity

The water holding capacity of soil samples was determined by the funnel method. Filter paper was placed in a funnel with about 20 g of soil. The funnel was placed into a beaker filled with water. The water was let to imbibe into the soil until the surface of the soil glistened brightly. The funnel was then removed from the beaker and drained for 30 minutes after which a spoonful

of soil was removed and placed on a tin pan. The pan and soil were weighed and placed into an oven maintained at 105° C for 24 h and weighed again.

Particle size analysis

The particle size distribution of the soil samples was determined using the pipet method (Gee and Bauder, 1986). Ten grams of < 2 mm, air dry soil was placed into flasks with 20 mL of DI water and 5 mL of hydrogen peroxide (H_2O_2) to oxidize organic matter. After 2 hours at room temperature, the samples were placed in a water bath maintained at 50° C and an additional 5 mL of H_2O_2 was added. After another 2 hours, the temperature of the water bath was increased to 95° C for one hour to decompose excess H_2O_2. The samples were then placed in an oven and dried overnight at 105° C. The next day, the samples were removed from the oven and placed in a desiccator, cooled to ambient temperature and the total weight of the samples was recorded.

After adding 10 mL of sodium-hexametaphosphate (NaHMP), equivalent to 0.4408 g of NaHMP, the samples were transferred to a 250 mL shaker bottle and the volume of the solution was brought to 175 mL with DI water. The bottles were placed in a horizontal shaker and shaken for 15 hours. The samples were then removed from the shaker and poured through a 300 mesh (0.047 mm) sieve placed in a funnel. The solution was collected in 1 L cylinders. The cylinders were filled to 1 L with DI water and allowed to equilibrate overnight. Sand retained on the sieve was washed into a beaker, dried at 105° C overnight, cooled in a desiccator, and weighed. The silt and clay fraction in the cylinders were stirred with a mechanical stirrer. After five hours, the clay fraction (< 2 μm) was determined in a 25 mL aliquot removed using a Lowy pipet. The aliquot was dried at 105° C overnight, cooled in a desiccator, and weighed. The silt fraction corresponds to the difference between the total weight and the sum of the sand and clay fractions.

pH/EC

Soil pH was measured in a 1:1 soil:water solution (Thomas, 1996). Soil and DI water were added to a 50 mL centrifuge tube and shaken for 15 min on a reciprocal shaker. Before measuring solution pH the, the samples were allowed to equilibrate for 30 min. The same solution was used to measure the electrical conductivity (Rhoades, 1996).

Total carbon and nitrogen

Total C and N of soil and plant samples were determined by the dry combustion method (Nelson and Sommers, 1996; Bremner, 1996). Subsamples were finely ground on a ball mill and weighed into tin cups. Total C and N were analyzed on a CNS analyzer NA 1500 series 2 (Carlo Erba Instruments, Milan, Italy).

Extractable nitrogen

Soil samples were weighed into centrifuge tubes, 2M KCl solution was added (KCl solution to soil ratio 5:1) and the tubes were shaken on a reciprocal shaker for one hour. After shaking, the

suspension was filtered through a filter paper (Fisherbrand, Q5; 12.5 cm diameter). Prior to use, the filter papers were leached with 15 mL of 2M KCl solution to remove any ammonium (Mulvaney, 1996).

The nitrate and ammonium concentrations in the filtrate were analyzed colorimetrically on a spectrophotometer (model UV mini 1240, Shimadzu). Nitrate was analyzed using a single reagent that reduces nitrate to nitrite which is complexed by sulfanilamide. Further reaction with N(1-naphthyl)ethylenediamine produces a red dye that is quantitated colorimetrically at 540 nm (Doane and Horwath, 2003).

The ammonium concentration was determined with the salicylate method (Verdouw et al. 1978). This method is based on the Berthelot reaction in which ammonium reacts with phenol and hypochlorite to form a green indophenol compound whose concentration is determined colorimetrically at 650 nm. The reagents used have been described by Foster (1995).

Following is a Table on soil characteristics used in this project.

Table 2. Properties of the soil samples used and past management.

Site		Carbon	Nitrogen	pH	EC	Texture				Crop	Cover crop	Animal
Location	ID	%	%		µS/cm	% Sand	% Silt	% Clay	Class	previous year	previous winter	Manure
Davis	bypass	1.13	0.13	8.03	171	2.7	55.4	41.9	silty clay	Sunflower	none	none
Davis	soccer	1.41	0.15	7.56	124	2.3	51.1	46.6	silty clay	Wheat	none	none
Woodland	D3	0.62	0.10	7.19	97	28.4	39.9	31.7	clay loam	Sunflower	none	none
Woodland	D4	0.72	0.10	6.94	141	33.3	39.8	26.9	clay loam	Sunflower	none	none
Artois	2005 Rd. 31	1.83	0.22	6.95	277	35.2	44.8	20.0	loam	Corn	cereal (for forage)	past years
Artois	2005 Rd. 30	1.51	0.16	6.66	182	22.2	58.7	19.1	silt loam	Corn	cereal (for forage)	past years
Stockton	W	1.77	0.18	7.28	180	17.3	40.1	42.5	silt clay	Tomatoes	none	none
Stockton	E	1.49	0.15	6.95	206	29.5	38.8	31.7	clay loam	Cotton	none	none
Stockton	N	0.53	0.06	6.98	92	80.9	13.8	5.3	loamy sand	Wheat	none	none
Lodi		1.81	0.12	7.64	164	54.7	30.2	15.1	sandy loam	Corn	cereal (for forage)	past years
LTRAS 05	Conventional	0.95	0.11	7.46	79	26.6	44.8	28.5	clay loam	Tomatoes	none	none
LTRAS 05	Organic	1.22	0.15	7.21	213	22.3	49.7	28.0	clay loam	Tomatoes	vetch	every year
LTRAS 05	Cover crop	1.08	0.12	7.30	150	21.0	49.9	29.1	clay loam	Tomatoes	vetch	none
Elk Grove	new	1.43	0.15	7.67	188	26.3	37.7	36.0	clay loam	Corn	cereal (for forage)	none
Elk Grove	old black	2.15	0.24	7.22	577	18.7	37.8	43.4	clay	Corn	cereal (for forage)	past years
Elk Grove	old red	0.97	0.12	7.33	508	44.5	39.9	15.5	loam	Corn	cereal (for forage)	past years
Elk Grove	red	0.88	0.10	7.31	73	44.3	39.9	15.8	loam	Corn	cereal (for forage)	none
Escalon	1	1.11	0.12	7.10	372	42.9	27.9	29.2	clay loam	Corn	cereal (for forage)	none
Escalon	2	2.10	0.19	6.89	234	47.5	31.8	20.7	loam	Pasture	cereal (for forage)	none
Escalon	3	0.84	0.09	7.09	442	44.3	32.2	23.5	loam	Pasture	cereal (for forage)	none
Artois	2004 W1	1.09	0.14	7.30	92	22.3	52.2	25.5	silt loam	Corn	cereal (for forage)	none
Artois	2004 No. 2	1.48	0.17	7.18	191	9.1	51.6	39.2	silty clay loam	Corn	cereal (for forage)	past years
Winters	W	0.68	0.10	7.02	95	33.4	38.1	28.5	clay loam	Tomatoes	none	none
Winters	E	0.59	0.09	6.96	138	25.8	42.0	32.2	clay loam	Sunflower	vetch	none
WSFS		0.78	0.10	7.57	331	22.0	40.1	37.9	clay loam	Wheat	none	none
LTRAS 04	Conventional	1.05	0.11	7.31	139	21.4	49.9	28.7	clay loam	Tomatoes	none	none
LTRAS 04	Organic	1.24	0.14	7.22	232	26.5	47.0	26.6	loam	Tomatoes	vetch	every year
LTRAS 04	Cover crop	0.94	0.11	7.30	156	27.3	49.4	23.3	loam	Tomatoes	vetch	none
Tulare		2.60	0.21	7.23	835	40.9	44.0	15.1	loam	Cotton	none	past years

Task 6, year 2. Identify additional responsive and non-responsive sites to N fertilization and collect soils.

and

Task 7, year 2. Characterize additional soils collected in year two physically and chemically.

We worked with Jeff Mitchell and Bruce Roberts to select a variety of fields containing different soil types and cropping histories. A description of sites and soil characteristics is given in Table 2 following the Task 2 summary.

Task 3, year 1. Determine potentially mineralizable N using established methods and test amino acid sugar assay. The methods include:

Pre-sidedress nitrate test (PSNT)

Composite samples of at least 5 cores were taken from the top 20 cm of soil, stored in an ice chest, dried in an oven at 40° C, and ground to pass a 2 mm sieve in order to allow for even mixing. The nitrate concentration was determined using the procedure described above.

Aerobic incubation

The aerobic incubation was carried out with air dry and sieved soil (Bundy and Meisinger, 1994). Water was added to reach either 60% water filled pore space or 60% water holding capacity determined by the funnel method. Samples were kept at 35° C in an incubator in the dark. The amount of soil and containers used, as well as the length of incubation depended on the experiment and will be described in the corresponding chapters. A separate sample was prepared for each sampling date to allow for destructive analysis of mineral N. Nitrate and ammonium were extracted with 2M KCl and analyzed colorimetrically as described for the analysis of mineral nitrogen. N mineralization was calculated by subtracting the mineral N at day zero from the mineral N determined after a certain incubation time. Periodic measurement of the mineralized N allowed for description of the relation between cumulative N mineralization (Nt) and time of incubation (t, in days) with the following equation based on first-order kinetics (Stanford and Smith, 1972):

$$Nt = N_0 (1\text{-}e\text{-}kt) \qquad \text{eq. 2.2}$$

Where N_0 is the N mineralization potential in units of mass (e.g. mg/kg of soil) and k is the specific rate of mineralization in units of reciprocal time (day^{-1}).

Anaerobic incubation

Soil samples were weighed into centrifuge tubes and 2.5 mL of DI water were added per gram soil. The tubes were purged with N2 to remove the oxygen in the headspace and closed immediately (Waring and Bremner, 1964). The samples were then placed in an incubator for

two weeks at 35° C. After removing them from the incubator, 2.5 mL of 4M KCl solution were added per gram soil and the samples were shaken on a reciprocal shaker for one hour, filtered through a pre-leached filter paper (Fisherbrand, Q5; 12.5 cm diameter) and analyzed colorimetrically for ammonium as described previously.

Soil amino sugar (Illinois Soil Test)

Amino sugars were analyzed using the direct diffusion method proposed by Khan et al. (2000, 2001). The method involves two analyses carried out with separate soil samples: Dried and crushed (2 mm) soil samples are treated in closed Mason jars either with a 2M KCl solution and MgO to convert ammonium-N to ammonia or with a 2M NaOH solution to convert ammonium-N and amino sugar-N to ammonia. A soil:solution ratio of 1:10 was chosen and 0.12 g MgO per g soil was added to the KCl solution. Five mL of boric acid (40 g H_3BO_3/L) contained in a Petri dish was suspended from the Mason jar lid. After the reagent additions, the jars were closed and placed on a hot plate (West Bend, model 76212) that was maintained at 48 to 50° C. The temperature of the plate was adjusted to heat 100 mL DI water in an open Mason jar, placed in the center or the griddle surface, to 48 to 50° C. The diffusion period was 5.5 hours to determine ammonium and 5 hours to determine amino sugar and ammonium. The liberated ammonia was collected in H_3BO_3 solution. After the diffusion period, the jars were allowed to cool before being opened. The boric acid solution was diluted with 10 to 15 mL of DI water and the Petri dishes containing the solution were weighed to take into account changes in weight during the diffusion period. The concentration of ammonium-N was determined colorimetrically (see above). The amino sugar content is the difference between the two results. Even though experiments showed that this N fraction can contain considerable amounts of N form other sources (Chapter 3), it will be called amino sugar in this thesis, as proposed by Khan et al. (2000, 2001).

Hot KCl

The hot KCl method was carried out as proposed by Gianello and Bremner (1986a), except that the samples were heated in a water bath instead of a block digester. Briefly, soil samples were weighted into glass vials (Fisherbrand, economical glass; 40 mL), 20 mL of 2M KCl was added per 3 g of soil and the vials were heated for four hours in a water bath maintained at 95 to 100° C. The solution was cooled for one hour after removing the vials from the water bath, and filtered through pre-leached filter papers (Fisherbrand, Q5; 12.5 cm diameter). The filtrate was analyzed colorimetrically for ammonium as described previously.

CO_2 evolution

Soil samples were weighed into septum equipped Mason jars or glass vials (Fisherbrand, economical glass; 40 mL) of known volume. Water was added to reach a water filled pore space of about 60%. The containers were closed and placed into an incubator maintained at 35° C. Headspace CO_2 was analyzed with a Qubit CO_2 analyzer (model S-151, Qubit Systems Inc., Kingston, Canada). After each analysis, the jars were opened and air exchange was forced using a bicycle pump. A blank was used to correct for background CO_2.

The ideal gas law was used to calculate the amount of organic carbon released based on the CO_2 concentration (Zibilske, 1994):

$PV = nRT$ eq. 2.3

Where P = Partial pressure of the gas (1% = 0.01 atm)
V = Volume of headspace in container (L)
n = Number of moles of gas
R = Gas constant (0.0821 L*atm*moles^{-1}*degree (K))
T = Temperature in Kelvin (°C + 273, 22° C = 295° K)

Solving the equation for n results in the moles of C released. The amount of carbon respired can then be calculated, as 1 mole of CO_2 contains one mole of carbon, which is equal to 12 g.

Microbial biomass C

The microbial biomass C was determined using the chloroform fumigation extraction method (Horwath and Paul, 1994). An air dry soil sample was weighed into a Mason jar. Water was added to reach a water filled pore space of about 60%. The samples were incubated at 35° C before being fumigated with chloroform for five days. Dissolved organic carbon (DOC) was extracted by adding 5 mL of 0.5M potassium sulfate (K2SO4) per gram soil, shaking the samples on a reciprocal shaker for 30 minutes and filtering the samples through a leached filter paper. The DOC in the filtrate was analyzed on a UV-Persulfate Total Organic C Analyzer (model Phoenix 8000, Tekmar DohrmannTM, Cincinnati, Ohio). Controls were treated identically except that they were not fumigated.

The microbial biomass C was calculated with the following equation (Horwath and Paul, 1994):

Biomass C = (Cf – Cuf)/Kec eq.2.4

Where Cf and Cuf are the carbon in the fumigated and unfumigated sample (control), respectively, and Kec is a correction factor equal to 0.35.

Statistical analyses

Statistical analyses were conducted with the SAS program (SAS Institute Inc., 1990), using the general linear model (GLM) procedure for analysis of variance and the REG procedure for regression analyses. When needed, data were transformed to meet the requirements of normality tested by the Shapiro Wilk test, homogeneity of variances (Levene's test), and additivity of the main effects (Tukey's test). Mean comparisons were performed using the REGWQ test. This multiple range test controls the experimentwise type I error rate (α) and has a low type II error rate and therefore a high power (Toothaker, 1991; SAS Institute Inc., 1990). Effects were considered significant at a p-value < 0.05. The NLIN (non-linear) procedure was used to fit exponential curves to data points.

Modifications of the direct diffusion method in Mason jars

Task 5, year 1. Modify amino sugar method to increase production efficiency for use in soil test labs.

The direct diffusion method is simple and convenient, it does not require specialized or expensive equipment, and the reagents are non-hazardous. Furthermore, the diffusion can be carried out overnight, as the results do not change when the jars are left closed for several hours at room temperature (chapter 3).
The previous chapter showed that the direct diffusion method as described is suitable for laboratory use, but that there are some limitations associated with the apparatus. The use of 1-pint mason jars requires a lot of space, and care must be taken when handling the Petri dishes, as the boric acid can easily be spilled. Another disadvantage of the method is that the jars can break at the base after multiple uses during the analysis presumably due to the large vertical temperature gradient. Finally, the unequal temperature reached in jars placed on different positions on the hot plate reduces the accuracy of the results.

Using smaller containers would reduce space requirements and increase production efficiency for use in soil test labs. A water bath could be used to heat the samples more evenly to reduce variable results. Two different approaches with the goal to miniaturize the method will be described and evaluated in this chapter after a brief discussion of the chemical principles of the direct diffusion method.

Principles of the direct diffusion method

The direct diffusion method involves two separate analyses: Soil samples are treated with a NaOH solution to convert ammonium-N and amino sugar-N to ammonia or with a KCl-MgO solution to convert ammonium-N to ammonia. The amino sugar content is the difference between the two results (Khan et al., 2000 and 2001).

The principle of the direct diffusion method with a NaOH solution is to release ammonium from amino groups of labile organic molecules through the action of elevated temperature and high pH. The method is quantitative for amino sugar and, when carried out with soil, only small amounts of N from other organic molecules, such as amino acids and amines are released (Mulvaney and Khan, 2001). At high solution pH, ammonium is converted to ammonia according to the following equation:

$$NH_4^+ + OH^- \leftrightarrows NH_3(aq) + H_2O$$

The reaction has a pKa of 9.5, which means that at a pH of 9.5, the concentrations of ammonium and ammonia in solution are equal. At a lower pH, ammonium is the dominant form; at a higher pH, ammonia predominates. At a pH of 7, approximately 0.36% of the total ammoniacal N in the soil solution is present as $NH_3(aq)$ (Nelson, 1982). As a 2M NaOH solution has a pH of 14.3, the above equation proceeds to the right. Aqueous ammonia is released into the headspace in relation to the partial pressure of gaseous ammonia. With the direct diffusion method, ammonia is

trapped in boric acid, where it is protonated to form ammonium. This reaction removes ammonia gas from the head space and maintains a gradient.

The principles of the extraction in a KCl-MgO solution are the same. The MgO increases the pH to about 10.2, so that most of the ammonical N is present as NH_3. Under the conditions of this extraction, only minor amounts of ammonium from organic molecules are released.
In order to maximize the release of ammonia into the headspace and trap it in the boric acid, the surface area of the solution and the acid trap have been maximized by using 1-pint Mason jars for the soil-reagent solution, and 60 mm diameter Petri dishes for the boric acid.

Materials and methods

Soil samples

The three methods were carried out with soil samples with a wide range of texture and organic matter content. The samples were taken form the top 20 cm of fields under row crops (a detailed description of the samples can be found in chapter 7). The samples were air dried and ground to pass a 2 mm sieve.

Standard direct diffusion method

The direct diffusion method has been described in chapter 2 and is the method of Khan et al. (2000, 2001). For the analysis presented in this chapter, 5 g of air dry soil and 50 mL of either 2 M NaOH or 2M KCl and 0.6 g MgO were used. At the end of the analysis, the boric acid was removed, sulfuric acid was added to duplicates of each sample and shaken for 15 minutes and then filtered. The solution of other duplicates was filtered directly. Both the filtrate and the boric acid were analyzed for ammonium.

Miniaturized diffusion method

This method uses the same principals as the direct diffusion method, except that the containers used are much smaller and the solution is heated in a water bath which allows uniform heating. Two grams of soil and 20 mL of 2M NaOH were added to a 40 mL glass vial (Fisherbrand, economical glass; 40 mL). A small plastic vial was placed into the glass vial and 3 mL of boric acid was added to the vial. A rubber band was wrapped around the top of the plastic vial to prevent condensed water on the inner wall of the glass vial from diluting the boric acid (Figure 2). The glass vials were closed immediately and placed in a water bath maintained at 50° C. After five hours the vials were removed from the water bath and cooled at room temperature for 30 minutes. The plastic vial containing the boric acid was then removed and the ammonium concentration in the acid was determined colorimetrically. To determine the exact amount of boric acid, the empty vials were weighed before use and the vials containing the boric acid were weighed again after the diffusion period. The analysis was carried out in triplicate.
In another experiment, the vials were left unopened for several hours at room temperature after the diffusion in the water bath to measure the N release over time. This experiment was carried out with NH_4Cl as N source.

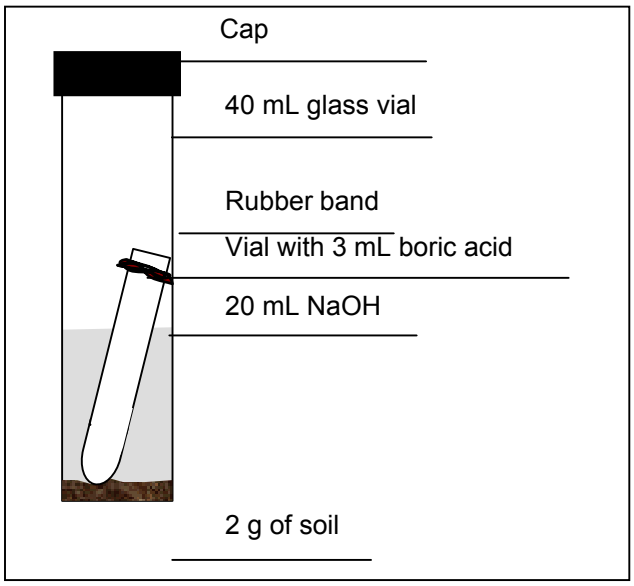


Figure 2. Design of the miniaturized direct diffusion method.

No-trap method

This method doesn't require an acid trap and can therefore be carried out in a small vial. The difference from the diffusion method is that at the end of the extraction period the pH of the whole solution is lowered to at least pH 7. The solution itself serves as an acid trap and can be analyzed for ammonium-N directly.

Two grams of soil were weighed into 40 mL glass vials (Fisherbrand, economical glass; 40 mL). Twenty mL of 2M NaOH was added and the vials were closed immediately using open-top caps with septa liners. About 3 mL of air was extracted with a syringe to lower the maximum pressure during the analysis. The vials were then placed into a preheated water bath maintained at 50° C. After five hours, the vials were removed from the water bath, and 3 mL of 9M H2SO4 was injected with a syringe to lower solution pH. The vials were shaken on a reciprocal shaker for 15 minutes to increase the rate of the conversion of ammonia to ammonium. The solution was then filtered through a previously leached filter paper. Finally, the ammonium-N in the filtrate was determined colorimetrically using a spectrophotometer. The analysis was carried out in triplicate. Two additional experiments were carried out. In one, the diffusion time was altered to study N release over time, in the other different amounts of acid were used.

The no-trap method was also carried out with 20 mL of 2M KCl and 0.2 g MgO to release ammonium-N. The procedure was the same as described for the NaOH extraction, except that the vials were left for 5.5 h in the water bath (Khan et al., 2001).

Results and discussion

Miniaturized diffusion method

The results for eight soils show that the N recovered by the miniaturized diffusion method was well correlated with the N recovered by the direct diffusion method in Mason jars (R^2 = 77%; Figure 3), but that the N recovery with the miniaturized method reached only about 5% of the N recovery with the direct diffusion method.

Two effects, surface to volume ratio of the soil-reagent solution and the boric acid solution and temperature of the boric acid, may have lead to the low recovery of the miniaturized method. The inner diameter of the glass vial and the plastic vial were small compared to a Mason jar and Petri dish. This may have lead to an incomplete release of ammonia from solution. This is consistent with the results from Mulvaney et al. (1997) who reported that recovery increased with jar diameter. Furthermore, ammonia in the headspace of the vial may not have been completely trapped by the boric acid. These assumptions are supported by the results of additional experiments in which NH_4Cl served as N source. After the diffusion period in the water bath, the vials were left at room temperature for different lengths of time before they were opened and the vial with the boric acid was removed.

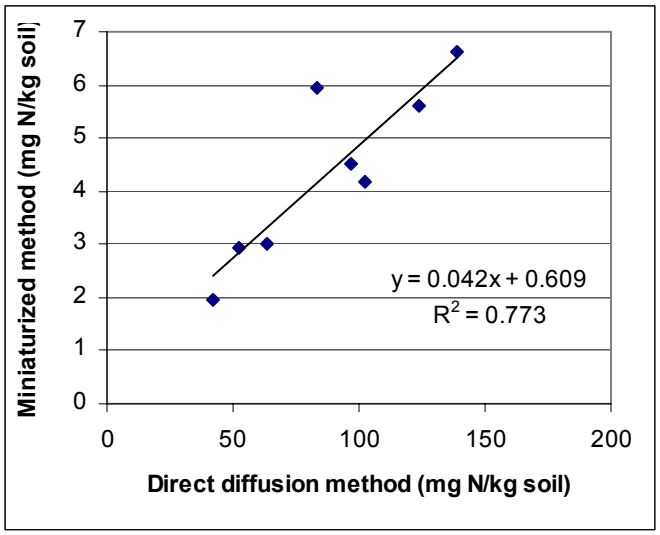


Figure 3. Correlation between the direct and the miniaturized diffusion method.

While 9.8% of the N was released during the diffusion period, recovery increased gradually and reached 21.8% after 24 h (Figure 4.3). The increased N recovery can be described with the following negative exponential equation:

$$Nt = N_0 + Nmax * (1\text{-}e\text{-}k*t).$$

Where Nt is the N released at time t after the end of the diffusion period, N_0 is the released N measured immediately after the end of the diffusion period, Nmax is the maximum increase, and k is a rate constant of release.

Substituting 0.098, 0.166, and 0.0532 for N_0, Nmax, and k, results in an expression that explains 99.7% of the variability. This result predicts that eventually 26.4% of the N would be released.

The recovery was higher when the miniaturized method was carried out with ammonium compared to soil. This may be due to the fact that the ammonium is dissolved in water and can be immediately converted to ammonia whereas labile organic N compounds have to be deaminated first. This may be the reason why one soil was relatively far off the regression line in the comparison between the direct diffusion method and the miniaturized method (Figure 4). This soil had a very high ammonium concentration compared to the amino sugar concentration.

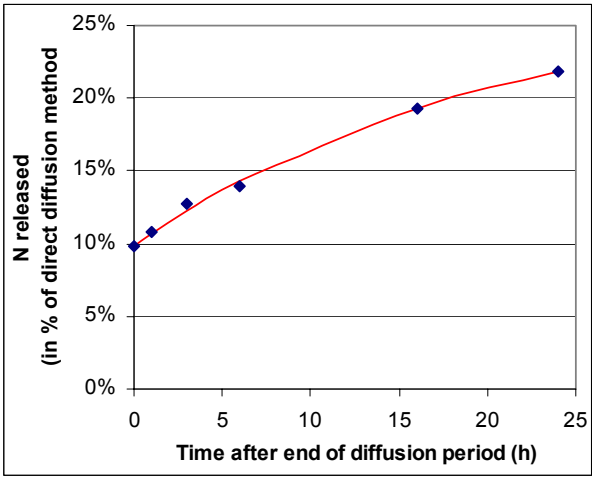


Figure 4. Recovery of N over time with the miniaturized diffusion method expressed in % of the N recovered with the direct diffusion method.

The lower recovery with the miniaturized method may also have been caused by a lowered NH3-absorption capacity of the boric acid due to the higher temperature. As the vial containing the boric acid was standing in the NaOH solution, it was heated to the same temperature. Mulvaney et al. (1997) found that the recovery was incomplete when diffusions were carried out in an incubator maintained at 35° C which they attributed to reduction in the capacity of boric acid for absorption of gaseous ammonia. The Mason jar approach avoids this problem by using a hot plate as a bottom heat source, thereby producing a temperature gradient within each jar (Khan et al., 1997).

No-trap method

The results of the direct diffusion method and the no-trap method were very well correlated (R^2 = 0.978; Figure 5). The no-trap method released significantly more ammonium than the direct diffusion method (p value < 0.0001).

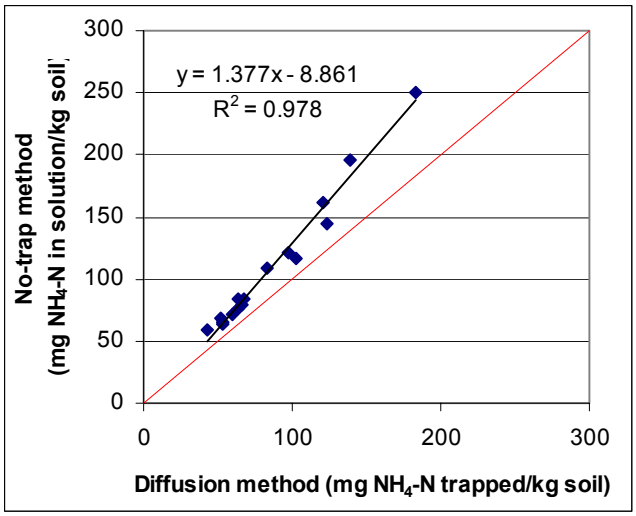


Figure 5. Correlation between soil amino sugar content measured by the direct diffusion method and the no-trap method.

Despite the fact that only 2 g of soil were analyzed with the no-trap method, compared to 5 g with the direct diffusion method, the no-trap method had a lower coefficient of variation (2.61% compared to 8.64%). The decrease in variability is most likely the result of the increased uniformity of temperature in the water bath compared to the hot plate.

The two methods differ in several ways: While the no-trap method measures all the ammonium in the solution, the standard diffusion method only measures the volatilized ammonia trapped in boric acid. Furthermore, with the new method, acid is added to lower the pH of the solution in order to dissolve the volatilized ammonia into solution and to convert ammonia to ammonium. The low pH may result in an additional release of ammonium from organic compounds.

The effects of these differences were tested by carrying out the direct diffusion method in Mason jars with eight soils. At the end of the analysis, the boric acid was removed and sulfuric acid was added to duplicates of each sample, which were shaken for 15 minutes and then filtered. The solution of the duplicate samples was filtered directly. Both the filtrate and the boric acid were analyzed for ammonium. A comparison between the ammonium in the acid trap and the ammonium remaining in solution shows that the amount of ammonium left in solution equals 56% of the ammonium trapped in boric acid (Table 4.1).

When acid was added to the solution, the ammonium in solution increased by 28%, to about 71% of the ammonium trapped in boric acid. Therefore, adding the ammonium found in the acidified solution to the ammonium trapped with boric acid reveals that the no-trap method releases less ammonium than the direct diffusion method (Figure 6).

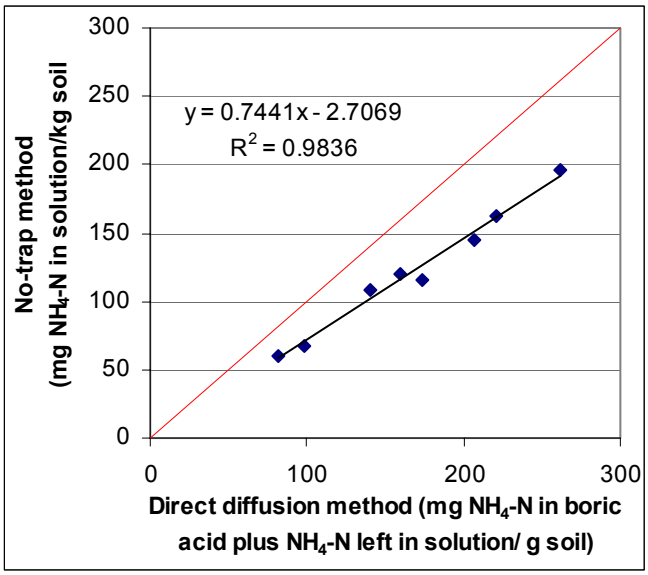


Figure 6. Comparison between total ammonium recovered by direct diffusion method and no-trap method.

The close correlation between the new method and the total ammonium recovered by the diffusion method and the small intercept of the regression line implies that the differences between the two results are caused by equilibrium reactions (Figure 6) resulting from different ammonia concentrations in the headspace during the extraction.

With the new method the ammonia in the headspace of the vial accumulates and may reach a high concentration, while with the diffusion method the ammonia is constantly removed by the boric acid. As equilibrium constants determine the ratio between the ammonium and the ammonia in solution as well as the ratio between ammonia in solution and volatilized ammonia, more ammonium is also likely to be present in the solution when the ammonia accumulates in the headspace. This may affect the deamination of amino sugars. Carrying out the direct diffusion method in Mason jars with the same eight soils as before, but without adding an acid trap tested this hypothesis. Instead, the jars were covered with a lid containing a septum. After five hours, 6.25 mL of 9M H_2SO_4 were added through the septum. The jars were then shaken for 15 minutes and the solution filtered and analyzed for ammonium.

The results show that 16% less ammonium was recovered when no acid trap was used compared to the total ammonium recovered when the ammonia concentration in the headspace of the jars was kept low by an acid trap (Table 3). The increasing ammonium concentration in the headspace and in the solution suppressed deamination of amino sugars.

The ammonium found in solution when no trap was present in the Mason jar was very well correlated with the no-trap method (R^2 = 96.7%) which uses the same principle, but smaller containers. The ammonium detected with the no-trap method was 13% lower (Table 3).

Table 3. NH_4-N recovered with different methods (values in mg N/kg soil).

	Diffusion method						No-trap method
		No acid added		acid added			
Soil #	in H_3BO_3	In solution	Total1)	In solution	Total1)	Without trap	
Fresno	86.95	35.46	122.41	53.12	140.07	122.62	108.39
Hickman	147.87	92.01	239.88	113.51	261.38	214.64	196.44
Artois 04 W1	92.55	61.03	153.59	66.52	159.07	147.46	120.66
LTRAS 04 org.	105.87	52.77	158.65	67.77	173.64	152.69	116.21
Winters E	59.99	29.74	89.74	38.64	98.64	82.09	67.95
Artois 04 No.2	121.98	67.23	189.21	84.39	206.37	155.31	145.27
WSFS	44.92	28.16	73.08	36.86	81.78	71.30	59.77
Tulare	123.61	69.13	192.75	97.25	220.87	182.76	162.10
Average	97.97	54.44	152.41	69.76	167.73	141.11	122.10
%	100%	56%	156%	71%	171%	144%	125%
					100%	84%	73%

1) The total is the sum of the NH_4-N in solution and in the boric acid.

The headspace in the mason jars is about 400 mL, but only about 20 mL in the vials. The ammonia concentration in the headspace inside the vials therefore reaches much higher levels, increasing the concentration of ammonium in solution, which in turn may slow the release of ammonium from amino sugars. This would explain the lower ammonium-N recovery with the no-trap method.

Release of amino sugar-N and ammonium-N over time with the no-trap method

The effect of increasing length of extraction on the release of amino sugar-N and ammonium-N was studied using three soils (Figure 7). The result shows that the extraction is not complete after 5 h, but proceeds further, releasing N from other organic molecules, presumably amino acids and amines. This finding is in line with the results obtained with the direct diffusion method (Khan et al. 2001). The N release as a function of time can be described with a quadratic equation (R^2 between 97 and 99%). The slope of the curve at any time depends on the soil sample analyzed. The slope is steeper for soils with high amino sugar concentrations compared to soils with low amino sugar concentrations. The time required until the amount of ammonium-N released is equal to the amount of ammonium-N trapped in the boric acid by the diffusion method differs from 1.3 h to 3.2 h and is not related to the total amount of ammonium-N released. A reduction of the extraction time would therefore not improve the agreement between the two methods.

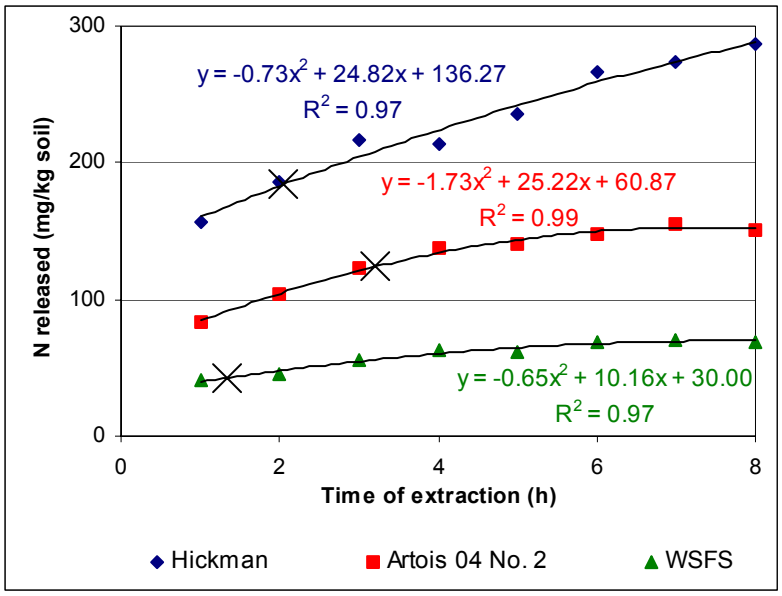


Figure 7. Release of amino sugar-N and ammonium-N by the no-trap method with increasing time of extraction (the x indicate the amount of ammonium-N determined with the standard diffusion method).

Determination of the ideal amount of acid to add

Without considering the effect of the soil on pH, 2.22 mL of 9M H_2SO_4 are needed to neutralize the pH of the 2M NaOH solution. The no-trap method was carried out with different amounts of acid to study the effect of the soil on the solution pH and to determine how much acid has to be added (Figure 8). As could be expected, the amount of soil was too small to have a significant pH buffering capacity. For both soils, 2.4 mL of sulfuric acid was enough to lower the pH below 2. The N measured did not change significantly when the acid addition was lowered from 3 to 2.2 mL, but was significantly lower for an addition of 2 mL. This is due to the high pH of about 12 in solution when only 2 mL of acid were added. Under these conditions, the ammonia was not converted to ammonium and was presumably lost when the vials were opened. An addition of 2.2 mL acid kept the pH low in one soil, but only decreased it to 8 in the other soil. In the second soil, the ammonium in solution was slightly lower than with a higher acid addition, but not significantly.

As the solution is poorly buffered around a neutral range, the use of a small excess of acid is recommended. Based on these results, an addition of 2.5 mL of acid to 20 mL 2M NaOH seems to be appropriate to lower the pH sufficiently.

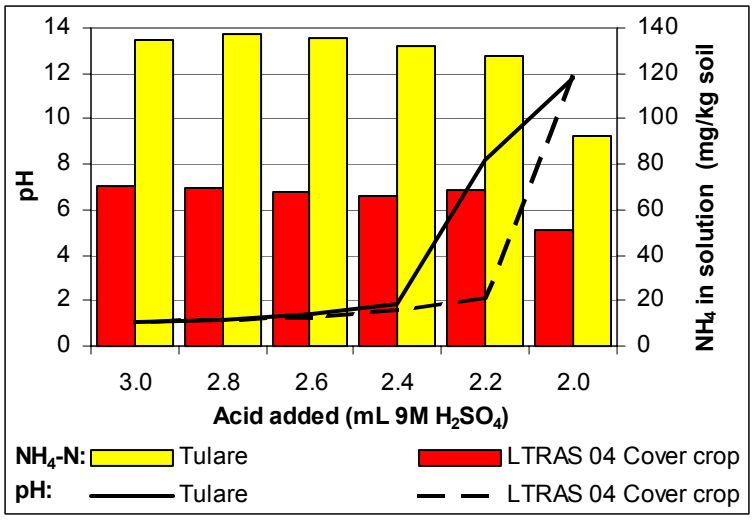


Figure 8. Ammonium in solution and pH with different amounts of acid added.

Exchangeable ammonium

The no-trap method was also compared with the direct diffusion method using a KCl-MgO solution to release exchangeable ammonium. With the direct diffusion method, the ammonium found in solution after the analysis was about 56% of the ammonium recovered with the acid trap. Taking the ammonium-N recovered in boric acid as 100%, the total ammonium-N recovered by the diffusion method was 156% without the addition of acid to the solution, and 189% with the addition of acid. The no-trap method recovered about 138%. The correlation between the N recovered in the acid trap of the diffusion method and the no-trap method was again close, resulting in an R^2 of 91%. Nevertheless, the results obtained with the no-trap method were less reliable because the MgO dissolved with the addition of acid and re-precipitated when the alkaline reagents for the color reaction were added. This resulted in cloudy solutions, some of which did not clear during the time the color was stable to be analyzed. Several samples could therefore not be analyzed with the spectrophotometer and only 12 of the 16 analyzed soils could be included in this comparison. The effect of the precipitate is also reflected in the higher CV, of 13.9% with the no-trap method compared to 8.4% with the diffusion method.

Amino sugar-N

Despite the difficulties in exchangeable ammonium-N determination, the amino sugar-N calculated for the diffusion method and the no-trap method are very well correlated (R^2 = 97.3%; Figure 9). The values obtained with the no-trap method were significantly higher (p-value < 0.0001). At very low amino sugar levels the results were comparable, but increase faster with the no-trap method as the concentration increases (slope of regression line = 1.36).

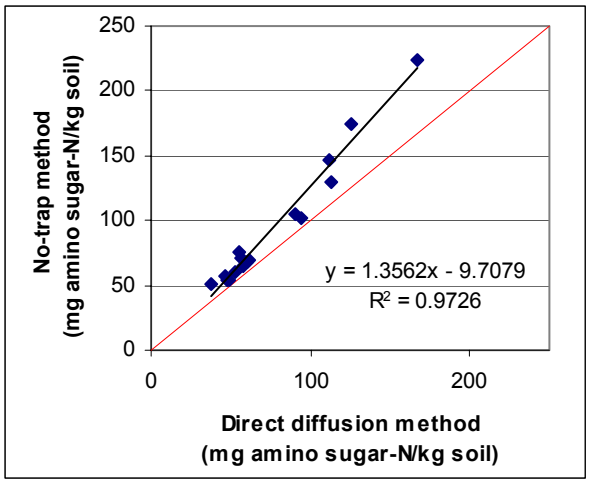


Figure 9. Amino sugar-N released with the diffusion method and the no-trap method.

Conclusions

A miniaturized version of the standard direct diffusion method of Khan et al. (2000; 2001) resulted in much lower measured soil amino sugar. This can be attributed to the much larger surface-to-volume ratios of the soil-reagent solution and the acid trap in the standard method and the resulting increase in the ammonia flux. Therefore, decreasing container size in the direct diffusion method is problematic. A second modification to the standard method - lowering solution pH after the diffusion period and retaining N as ammonium in the soil-reagent solution - seems to be a more promising approach which does not depend on the size of the containers used.

The direct diffusion method recovers amino sugar-N almost completely (Mulvaney and Khan, 2001). Only a minor part of the N released originates from other N sources such as amino acids and amines. With the no-trap method, more N is recovered because the ammonium-N, which remains in solution, is measured too. Therefore the proportion of N from amino sugar is supposed to be lower, since ammonium from other sources contributes more to the measured N. Nevertheless, the two methods are very well correlated. The no-trap method has several advantages over the diffusion method, which make it attractive for use in soil test labs: The variation could be significantly decreased by eliminating the effect of the unequal temperature reached in the jars during the extraction. Furthermore, the no-trap method can be carried out without using bulky mason jars, and all the material used is designed for laboratory use and doesn't need to be adapted for this analysis. While several jars broke during the analysis, no vial broke in the water bath.

Nevertheless, the new method has also its disadvantages: One more step (filtration) is required, the ammonium cannot be determined by titration; and the acid used is very strong, requiring careful handling. The application of the acid, however, can be simplified by using a self-refilling repetitive syringe. Furthermore, the new method doesn't allow for ^{15}N analysis and is not suitable for the determination of ammonium-N to calculate the amino sugar-N. Nevertheless, this last point is not necessarily a disadvantage, as results from our lab showed a closer correlation between aerobic incubation and the N determined with NaOH than with the amino sugar content

(see next section). The advantages and disadvantages of the new method are summarized in table 4. The no-trap method could be improved by using taller vials, which allow addition of a larger volume of acid with a lower concentration.

Table 4. Advantages and disadvantages of the now-trap method compared to the direct diffusion method.

Advantages	Disadvantages
Much less lab space required	Solution has to be filtered
More samples can be analyzed at a time	Ammonium-N determination cannot be done by titration
No easily spilled solution in Petri dishes needed	Strong acid needed to lower pH
Temperature can be controlled more easily	Doesn't allow for 15N analysis
Lower variation between subsamples	Doesn't allow for determination of exchangeable ammonium, due to formation of precipitate
Material needed is designed for lab use	
No adaptation of material needed	
Vials do not break during analysis	

Field Evaluation of Chemical and Biological Indices for Soil N Mineralization

The following tasks are addressed in this section.

Task 4, year 1. Characterize the fertilizer N responsiveness of sites.

Task 8, year 2. Determine potentially mineralizable N using established methods and test amino acid sugar assay on soils collected in the second year.

Task 9, year 2. Characterize the fertilizer N responsiveness of sites.

Task 11, year 3. Continue soil testing and finalize standard operating procedure for amino sugar method and develop value and sliding scale approach for the interpretation of the assay.

Many productive mineral soils contain several thousand kilograms of N per hectare, most of which is unavailable for plants in the form of organic matter (Foth and Ellis, 1997). The mineralization of only a small part of the organic N during the cropping season can provide a significant proportion of the N needed by crops (Hadas et al, 1986). The application of N fertilizer without taking into account the soil mineralization potential can therefore lead to inefficient use of N and an increased risk of groundwater pollution with nitrate.

There have been many attempts to find an accurate chemical or biological index of soil N availability that could be used to improve N fertilizer recommendations (Chapter 1). None of these tests has found widespread acceptance for row crops and other systems. For this reason, farmers rely mainly on their experience from preceding years to estimate crop N demand. While fertilization based on previous yields may prevent under-application of fertilizer, it is less successful at determining over-application of N. A reliable N fertilizer recommendation which takes into account the N supplying capacity of a soil would reduce excess application of N and lead to the development of efficient fertilizer application.

The objective of the present study was to evaluate the correlation between the most promising soil tests presented in the introduction, including the direct diffusion method. The evaluation of the methods was done in two steps: First, the soil tests were compared with the results of an aerobic incubation, which is generally recognized as a sound relative measure of the mineralization potential of a soil. Second, the different tests were compared with the N uptake of corn and corn response to N fertilization in the field. This two-step approach was chosen because many factors, such as weather pattern and crop management, affect crop development in the field. These factors may mask a good correlation between a soil test and N mineralization.

Material and methods

Soil sampling

Soil samples were taken in spring from the top 20 cm when the corn had between 2 and 7 leaves (stage V2 to V7). A composite sample of at least five cores was taken from each plot. The soil samples were dried at 40° C and crushed to pass a 2 mm sieve.

Aerobic incubation

Dried and sieved soil (8 g) was weighed into a centrifuge tube and moistened with DI water to reach 60% water filled pore space. Five such centrifuge tubes were placed in a Mason jar containing 30 mL of DI water to keep the humidity high and prevent evaporation from the soil sample. The Mason jars were closed and placed into an incubator maintained at 35° C. The jars were opened regularly and air exchange was forced with a bicycle pump. After 7, 14, 21, 35, 49, and 70 days, nitrate and ammonium were extracted from randomly selected tubes with 40 mL of 2M KCl and determined colorimetrically (for further details see chapter 2).

The cumulative N mineralized during the aerobic incubation was analyzed in four different ways:

- N_0;0-70: Cumulative net N mineralization between days 0 and 70.
- N_0;7-70: Cumulative net N mineralization between days 7 and 70. This approach was chosen because the flush of N mineralization that follows rewetting a dry soil may release significant amounts of N, which may lead to an over-estimation of the real N mineralization potential (Stanford and Smith, 1972; Cabrera, 1993; Bundy and Meisinger, 1994; Cabrera and Kissel, 1988).
- N_0;0-70: The cumulative N mineralized (Nt) between days 0 and 70 was modeled using a single exponential equation, proposed by Stanford and Smith (1972):
- $Nt = N_0 (1-e-kt)$
- Where N_0 is the N mineralization potential, k is the specific rate of mineralization, and t the time of incubation in days.
- N_0;7-70: N_0 and k were calculated with the same equation, but without the N mineralized during the initial 7 days of incubation.

Different mathematical models have been proposed to analyze cumulative N mineralization (Molina et al., 1980; Juma et al., 1984). However, few authors have fully explained the theoretical implications of the parameters in these mathematical equations (Ellert and Bettany, 1988). Single and double exponential equations remain the most widely used equations. For this study, the single exponential equation has been chosen, because is has been found to describe cumulative N mineralization well during short-term incubations of less than 15 weeks (Dou et al., 1996).

The soil N mineralization potential (N_0) and the rate constant (k) were determined from the cumulative N mineralized using the SAS NLIN (non-linear) procedure. The proc REG procedure was used to generate the correlation matrixes (SAS Institute Inc., 1990).

Laboratory methods

The following methods to assess N mineralization were carried out and compared with the results of the aerobic incubation and crop N uptake in the field:

- Pre-sidedress nitrate test (Magdoff et al., 1984)
- Direct diffusion method for amino sugar and ammonium (Khan et al., 2000 and 2001)
- Direct diffusion method for amino sugar (Khan et al., 2000 and 2001)
- No-trap method (described earlier)
- Anaerobic incubation (Waring and Bremner, 1964)
- Hot KCl method (Gianello and Bremner, 1986a)
- Total C and N determined by dry combustion (Nelson and Sommers, 1996)
- Short-term CO2 evolution (Franzluebbers et al., 2000)

These methods as well as the methods used to characterize the soil samples are described in detail in chapter 2.

Fertilization trials

The response of corn to N fertilization was determined in 2004 and 2005 in plots at UC Davis' Long-Term Research on Agricultural Systems (LTRAS) site and at other field sites in the northern Central Valley, CA. Field sites were chosen to represent a wide range of soil properties and crop management (Table 5). In spring after planting, three plots (6 rows wide and 30 feet long) were marked with flags. The plots were located at least 15 m from the end of the field and six rows (4.5 m) from the field edge. The corn in these plots did not receive any N fertilizer after emergence but in most cases received a small amount of N in the form of starter fertilizer when planted. Starter fertilizer also contained P, K, and micronutrients.

The corn was harvested as silage corn (R5 stage; dent) when the plants had a dry matter content of about 30%. Plants were harvested by hand from an area of 4 m x two rows in the center of each plot. Three additional plots of the same size were harvested in the fertilized area adjacent to the control plots. The plants were weighed in the field and a subsample of 8 to 10 plants per plot was chopped with a garden shredder/chopper (Craftsman; model no. 247.776350). The chopped material was mixed thoroughly, about half of it placed in a paper bag, weighed and dried at 60° C to determine the dry matter content of the corn. The subsample was then ground with a Wiley mill to pass a 2 mm screen. The ground material was thoroughly mixed and a subsample was ball milled and analyzed for total C and N by dry combustion (described earlier). The laboratory and field data were used to calculate the corn N uptake in unfertilized and fertilized plots. N uptake was defined as the total N in the aboveground biomass of the corn plants. To correct for the starter N, an amount of N equal to 75% of that applied as starter fertilizer was subtracted from the corn N uptake.

The N uptake in unfertilized plots is a good estimate of the N mineralization from soil organic matter and crop residues under the assumptions that these plots did not receive any additional nitrogen, that no N was lost or moved from the root zone during the cropping season, and that other factors (e.g., pests, non-N nutrient deficiencies, weather, or irrigation mistakes) did not limit growth and N uptake. A potentially significant source of additional N may be the irrigation water. For example, the application of 90 cm of water during the cropping season with a nitrate-N concentration of 1 ppm results in a N input of 9 kg/ha. Therefore, water with a nitrate-N

concentration of 10 ppm, which is the maximum contaminant level for drinking water in California (Anton et al., 1988), results in an input of 90 kg N/ha. To account for additional N inputs, irrigation water was analyzed for its nitrate content.
The response of corn to N fertilization was calculated with the following equation:

$$\text{N response} = \frac{\text{(Yield/N uptake fertilized plots - Yield/N uptake unfertilized plots)}}{\text{Yield/N uptake unfertilized plots}}$$

This approach is based on the assumption that the fertilized corn reaches a maximum yield and N uptake, so that further fertilization would not have any effect. Furthermore, as with the N uptake, the unfertilized plots should not receive any additional N.

Table 5. Properties of the soil samples used and past management.

Site		Carbon	Nitrogen	pH	EC	Texture				Crop	Cover crop	Animal
Location	ID	%	%		µS/cm	% Sand	% Silt	% Clay	Class	previous year	previous winter	Manure
Davis	bypass	1.13	0.13	8.03	171	2.7	55.4	41.9	silty clay	Sunflower	none	none
Davis	soccer	1.41	0.15	7.56	124	2.3	51.1	46.6	silty clay	Wheat	none	none
Woodland	D3	0.62	0.10	7.19	97	28.4	39.9	31.7	clay loam	Sunflower	none	none
Woodland	D4	0.72	0.10	6.94	141	33.3	39.8	26.9	clay loam	Sunflower	none	none
Artois	2005 Rd. 31	1.83	0.22	6.95	277	35.2	44.8	20.0	loam	Corn	cereal (for forage)	past years
Artois	2005 Rd. 30	1.51	0.16	6.66	182	22.2	58.7	19.1	silt loam	Corn	cereal (for forage)	past years
Stockton	W	1.77	0.18	7.28	180	17.3	40.1	42.5	silt clay	Tomatoes	none	none
Stockton	E	1.49	0.15	6.95	206	29.5	38.8	31.7	clay loam	Cotton	none	none
Stockton	N	0.53	0.06	6.98	92	80.9	13.8	5.3	loamy sand	Wheat	none	none
Lodi		1.81	0.12	7.64	164	54.7	30.2	15.1	sandy loam	Corn	cereal (for forage)	past years
LTRAS 05	Conventional	0.95	0.11	7.46	79	26.6	44.8	28.5	clay loam	Tomatoes	none	none
LTRAS 05	Organic	1.22	0.15	7.21	213	22.3	49.7	28.0	clay loam	Tomatoes	vetch	every year
LTRAS 05	Cover crop	1.08	0.12	7.30	150	21.0	49.9	29.1	clay loam	Tomatoes	vetch	none
Elk Grove	new	1.43	0.15	7.67	188	26.3	37.7	36.0	clay loam	Corn	cereal (for forage)	none
Elk Grove	old black	2.15	0.24	7.22	577	18.7	37.8	43.4	clay	Corn	cereal (for forage)	past years
Elk Grove	old red	0.97	0.12	7.33	508	44.5	39.9	15.5	loam	Corn	cereal (for forage)	past years
Elk Grove	red	0.88	0.10	7.31	73	44.3	39.9	15.8	loam	Corn	cereal (for forage)	none
Escalon	1	1.11	0.12	7.10	372	42.9	27.9	29.2	clay loam	Corn	cereal (for forage)	none
Escalon	2	2.10	0.19	6.89	234	47.5	31.8	20.7	loam	Pasture	cereal (for forage)	none
Escalon	3	0.84	0.09	7.09	442	44.3	32.2	23.5	loam	Pasture	cereal (for forage)	none
Artois	2004 W1	1.09	0.14	7.30	92	22.3	52.2	25.5	silt loam	Corn	cereal (for forage)	none
Artois	2004 No. 2	1.48	0.17	7.18	191	9.1	51.6	39.2	silty clay loam	Corn	cereal (for forage)	past years
Winters	W	0.68	0.10	7.02	95	33.4	38.1	28.5	clay loam	Tomatoes	none	none
Winters	E	0.59	0.09	6.96	138	25.8	42.0	32.2	clay loam	Sunflower	vetch	none
WSFS		0.78	0.10	7.57	331	22.0	40.1	37.9	clay loam	Wheat	none	none
LTRAS 04	Conventional	1.05	0.11	7.31	139	21.4	49.9	28.7	clay loam	Tomatoes	none	none
LTRAS 04	Organic	1.24	0.14	7.22	232	26.5	47.0	26.6	loam	Tomatoes	vetch	every year
LTRAS 04	Cover crop	0.94	0.11	7.30	156	27.3	49.4	23.3	loam	Tomatoes	vetch	none
Tulare		2.60	0.21	7.23	835	40.9	44.0	15.1	loam	Cotton	none	past years

Table 6. Results of different nitrogen mineralization indices (n=3).

Site		Amino sugar	Amino sugar & NH_4	No-trap	Anaerobic	Hot KCl	PSNT	$N_{0\text{-}70}$ [1)]	Total N	Total C	CO_2 flush
Location	ID	---------- (mg N/kg dry soil) ----------							--- (%) ---		(mg C/kg dry soil)
Davis	bypass	73.7	83.6	89.3	10.1	13.5	15.2	42.8	0.127	1.13	109.4
Davis	soccer	109.3	122.8	109.6	22.3	7.2	22.9	49.3	0.153	1.41	128.9
Woodland	D3	68.6	86.2	85.2	1.2	8.3	24.5	27.1	0.096	0.62	80.7
Woodland	D4	62.9	77.0	80.6	6.5	9.9	25.7	25.0	0.097	0.72	78.1
Artois	2005 Rd. 31	167.2	181.1	185.7	31.6	15.7	38.0	137.8	0.217	1.83	294.8
Artois	2005 Rd. 30	127.7	139.8	144.5	26.9	11.0	18.1	91.5	0.160	1.51	184.2
Stockton	W	130.6	147.0	123.2	8.3	7.0	27.3	37.4	0.180	1.77	153.5
Stockton	E	96.5	106.0	129.3	10.2	11.8	18.8	49.1	0.145	1.49	140.5
Stockton	N	36.2	41.7	52.6	14.3	6.0	10.7	43.7	0.059	0.53	106.6
Lodi		89.5	97.9	105.6	7.6	8.7	17.7	60.3	0.124	1.81	147.9
LTRAS 05	Conventional	69.7	77.9	61.7	8.8	4.0	13.5	32.2	0.107	0.95	98.0
LTRAS 05	Organic	103.9	112.4	86.3	19.8	8.2	27.8	60.6	0.145	1.22	127.6
LTRAS 05	Cover crop	75.3	83.7	75.1	16.9	9.7	21.0	39.8	0.118	1.08	107.3
Elk Grove	new	150.8	175.6	153.9	10.6	19.2	27.2	66.0	0.147	1.43	232.4
Elk Grove	old black	169.7	203.1	199.1	38.1	16.1	69.3	104.5	0.238	2.15	244.4
Elk Grove	old red	95.2	124.4	143.4	6.6	15.7	66.0	115.0	0.121	0.97	234.2
Elk Grove	red	100.8	114.6	116.5	5.7	9.4	13.6	54.8	0.103	0.88	185.5
Escalon	1	92.6	109.1	115.2	32.8	5.0	27.0	90.8	0.116	1.11	263.0
Escalon	2	170.5	202.7	236.4	66.2	8.9	42.0	129.2	0.189	2.10	419.7
Escalon	3	74.5	90.0	107.3	5.6	5.1	30.5	71.8	0.093	0.84	192.1
Artois	2004 W1	94.3	102.5	105.8	21.6	7.4	7.0	109.3	0.136	1.09	249.4
Artois	2004 No. 2	128.9	142.6	135.4	26.0	9.6	17.4	109.8	0.166	1.48	275.5
Winters	W	75.1	86.0	78.2	8.1	4.8	17.1	31.5	0.097	0.68	111.6
Winters	E	58.3	66.4	64.6	11.3	5.3	16.3	35.9	0.087	0.59	144.9
WSFS		52.2	58.5	64.0	16.6	2.8	20.7	59.7	0.096	0.78	104.7
LTRAS 04	Conventional	76.7	85.8	78.0	18.0	3.6	13.0	52.1	0.112	1.05	166.2
LTRAS 04	Organic	98.5	107.3	103.3	35.6	6.1	20.3	95.8	0.144	1.24	239.1
LTRAS 04	Cover crop	61.5	70.5	69.0	25.2	4.4	17.0	44.7	0.107	0.94	156.5
Tulare		147.6	160.2	172.5	19.6	13.6	164.4	168.4	0.206	2.60	203.4

[1)] Cumulative N mineralized during a 70-day aerobic incubation.

Results and discussion

Aerobic N mineralization and N mineralization indices

The nitrogen mineralized during the 70-day aerobic incubation ranged from 25.0 to 168.4 mg N/kg dry soil (Table 6). Total soil N and N mineralized were significantly correlated (p-value < 0.0001), but the correlation was only moderate (R^2 = 0.476). Between 2.1% and 9.5% of the total soil N was mineralized. This percentage was higher for soils with a high net N mineralization, which may be due to the fact that net N mineralization was highest in fields with a history of animal manure application or high organic residue input (cover crop, use as pasture). These organic materials may have a larger proportion of readily available compounds than the residual soil organic matter. Organic amendments therefore have a positive effect on both the proportion of N mineralized and the total amount of N mineralized. The proportion of N mineralized decreased significantly with increasing clay content (p-value = 0.0024). Nevertheless, the correlation between clay content and proportion of N mineralized was weak (R^2 = 0.295), indicating that other factors also affect the availability of N. There was no significant correlation between the C/N-ratio of the soil organic matter and the N mineralized.

For most soils, the cumulative N mineralized could be described very well with the negative exponential equation proposed by Stanford and Smith (1972). The R^2-values ranged from 94% to 99.8% (Figure 10).

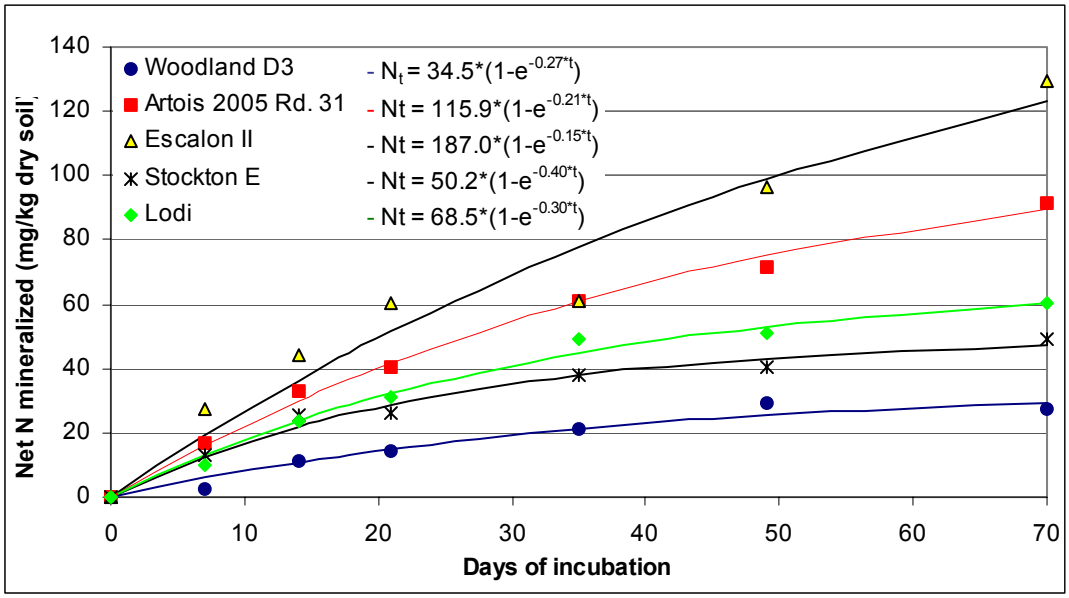


Figure 10. Cumulative N mineralization measured during 70 days of aerobic incubation (points) and modeled (line) from selected soil samples.

For some soils the program failed to converge. These fields were not included in the comparison. The difficulties were more pronounced when the N mineralized during the first week was excluded.

On average, the calculated N mineralization potential (N_0) exceeded the cumulative N mineralized during 70 days of incubation by about 35%. This indicates that most of the readily available N has been mineralized during the incubation period. The two values were well (R^2 = 0.91) and significantly (p-value < 0.0001) correlated.

An increase in the specific mineralization rate (k) results in a more convex curve, which means that a larger proportion of the nitrogen is mineralized in the early stages of the incubation. One would therefore expect to find lower k values in soils where high clay or soil organic matter contents could protect organic compounds from being decomposed. However, no significant correlation between k and clay content, total C and N and the C/N ratio of the organic matter were found.

All the N availability indices were positively correlated with the N mineralized during ten weeks of aerobic incubation (Table 7.3), and were highly significant. Nevertheless, the variability of the data was large, resulting in moderate agreement between the different indices and the N mineralized. The R2 values, which are a measure of the proportion of the total variability explained by the linear regression, ranged from 0.20 to 0.65. The index most closely related to net N mineralization was the three-day CO2 flush. This is in agreement with Franzluebbers et al. (2000) who found a good correlation between the flush of CO2 after rewetting dried soil and the net N mineralization during 24 days. This result clearly shows how closely the cycles of carbon and nitrogen are linked in soils, but it is surprising to see such a close correlation between a short term CO2 flush, highly related to the effects of sieving and drying the soil samples, and the N mineralized during ten weeks. The other methods were no better or even less well related to N mineralization than the soil total C and N content.

Excluding the N mineralized during the first 7 days slightly worsened the correlation between the N mineralized aerobically and the all of the N availability indices. The same is true for the use of a negative exponential equation to calculate the N mineralization potential. The measured net N mineralization was with few exceptions better correlated with the different N mineralization indices than the calculated N_0 (Table 7).

Table 7. Pearson correlation coefficients (r) and p-values for the correlations between N mineralized during an aerobic incubation and different N availability indices.

N availability index	Aerobic incubation			
	N0-70	N7-70	N_0;0-70	N_0;7-70
Number of observations	29	29	24	19
PSNT	0.652	0.644	0.356	0.504
	0.0001	0.0002	0.0874	0.0278
Direct diffusion for amino sugar	0.703	0.666	0.526	0.593
	<.0001	<.0001	0.0082	0.0074
Direct diffusion for amino sugar & NH_4^+	0.689	0.654	0.536	0.619
	<.0001	<.0001	0.0069	0.0048
No-trap method	0.770	0.735	0.634	0.707
	<.0001	<.0001	0.0009	0.0007
Hot KCl	0.443	0.451	0.210	0.641
	0.0161	0.0142	0.3239	0.0031
Anaerobic incubation	0.602	0.505	0.643	0.301
	0.0006	0.0052	0.0007	0.2101
CO2 flush (3 days)	0.803	0.777	0.871	0.844
	<.0001	<.0001	<.0001	<.0001
Total C	0.686	0.627	0.369	0.352
	<.0001	0.0003	0.0756	0.1397
Total N	0.690	0.631	0.392	0.383

Relationship between soil amino sugar and other N mineralization indices

The amino sugar content was highly significantly (p-value < 0.0001) and very well correlated with the no-trap method (R^2 = 0.87), which is in agreement with the results presented in chapter 4. Soil amino sugar, determined by the direct diffusion method, was also well related to the total soil N (R^2 = 0.86), which supports the results from the UC Davis Long-Term Research on Agricultural Systems site summarized in chapter 5. No other method, including total C, was closer related with total N than soil amino sugar. The correlations between soil amino sugar and total carbon (R^2 = 0.83), aerobic incubation (R^2 = 0.48), anaerobic incubation (R^2 = 0.33), hot KCl method (R^2 = 0.35), and CO2 flush (R^2 = 0.36) were lower, but still highly significant (p-values < 0.001). A very weak but still statistically significant correlation was observed between the amino sugar content and the PSNT and C/N-ratio of the soil organic matter. All these correlations were positive, which means that an increase in soil amino sugar was associated with an increase in the other variable. These results changed only slightly when the ammonium was not subtracted from the N released during the direct diffusion method with NaOH.

The SAST was slightly better correlated with the N mineralized aerobically than the amino sugar content (R^2=0.59).

Corn growth in the field and N mineralization indices

The corn yield in the unfertilized plots varied widely between sites, while the yield obtained in the fertilized plots covered a much smaller range (Table 8), which may indicate that the yield in the fertilized plots was close to its potential.

In general, N fertilization increased the plant N concentrations. Therefore, the response to N fertilization was more pronounced when aboveground N uptake rather than yield was compared. The corrected N uptake in unfertilized plots (N uptake – starter-N*0.75), a measure for the N mineralization potential of a site, ranged from 45 to 319 kg N/ha.

As mentioned above, the validity of the comparison between soil N mineralization and N uptake relies on the assumption that the unfertilized crop did not receive any additional N. The calculation of the fertilizer response depends on this assumption as well, but also requires that the fertilized plants reached their growth maximum. As discussed below, these assumptions were clearly not met in three fields.

Table 8. Yield and N uptake of corn grown in the field.

	Site	Yield (t dry wt./ha)		N uptake (kg/ha)		
Location	Field	unfertilized	fertilized	unfertilized	ilized	corrected [1]
Davis	bypass	17.7	21.0	134.7	269	125
Davis	soccer	20.8	22.5	222.3	325	213
Woodland	D3	16.6	22.6	143.4	296	134
Woodland	D4	16.7	17.3	239.0	258	230
Artois	2005 Rd. 31	21.7	20.5	318.6	309	319
Artois	2005 Rd. 30	15.1	17.9	161.4	189	161
Stockton	W	16.1	16.4	166.0	168	157
Stockton	E	15.9	18.6	194.1	268	185
Stockton	N	18.4	17.2	247.9	279	248
Lodi		20.9	23.9	164.3	260	147
LTRAS 05	Conventional	14.9	23.6	121.9	288	90
LTRAS 05	Organic	16.6	16.2	140.9	126	141
LTRAS 05	Cover crop	13.9	20.8	111.7	227	112
Escalon	1	20.0	22.3	212.1	269	199
Escalon	2	15.8	16.8	196.8	235	184
Escalon	3	16.2	17.3	199.5	211	187
Artois	2004 W1	9.2	16.7	57.4	114	45
Artois	2004 No. 2	19.1	20.6	175.7	207	176
Tulare		26.1	26.7	334.1	332	321
LTRAS 04	Conventional	18.8	27.2	136.5	265	105
LTRAS 04	Cover crop [2]	15.0		95.8		96

1) N uptake unfertilized minus 75% of N added with starter fertili

2) No fertilized plots established

The NO_3-N concentration in the irrigation water for field Stockton N was 15.8 ppm. Using an average application of 0.9 m^3/m^2 for the whole season and assuming a constant nitrate concentration, this nitrate concentration resulted in an N input of about 140 kg/ha in the

unfertilized plots. Therefore, this field was excluded from the comparison. In the other fields, the nitrate concentration in the irrigation water was much lower, resulting in N inputs of less than 30 kg/ha for the whole season. As the nitrate concentration in the irrigation water could not be monitored during the whole season, no correction was made for the N input with irrigation water.

In 2005, the N uptake of the unfertilized corn in the conventional, cover crop, and organic treatments at LTRAS was similar. The fertilization with mineral fertilizer increased the N uptake considerably in the conventional and cover crop treatments, however the application of animal manure did not increase the yield in the organic treatment. The N uptake in the fertilized organic plots was not significantly higher than in the unfertilized plots of any treatment (Table 7.4). This indicates that N was a limiting factor for the fertilized organic corn. Therefore, the organic corn can be used for the comparison between soil tests and N uptake in unfertilized plots, but will not be included in the comparison between soil tests and response to N fertilization.

The two sites in Woodland were located in adjacent fields of the same farm and were managed similarly during the previous years (Table 5). The soil properties were very similar as well (Table 5). Nevertheless, while in field D3 the response to N fertilizer was large, no such response could be seen in field D4, where the corn N concentration and N uptake in the "unfertilized" plots almost reached the level of the fertilized plots. While the N concentration of the corn in both the fertilized and unfertilized plots of field D4 was significantly higher than in the fertilized plots of field D3, the total N uptake was lower. This suggests that N was added to field D4 later in the season after sidedressing. Therefore, field D4 was excluded from the comparison.

A total of 17 fields were included in the comparison between soil tests and crop response to N fertilization, and 19 fields were included in the comparison between soil tests and N uptake in unfertilized plots.

The 70-day aerobic incubation was moderately well correlated with the corrected N uptake in unfertilized plots ($R^2 = 0.41$; p-value = 0.0033). Excluding the first week only slightly improved the agreement with the field data (Table 9). The calculated N mineralization potential (N_0) was not significantly related to the corn N uptake. This may be at least partly due to the fact that the cumulative N mineralization from some soils could not be expressed with the exponential equation.

Table 9. Pearson correlation coefficients (r) and p-values for the correlations between N mineralization indices and N uptake as well as response to N fertilization.

N availability index		Corrected N uptake		Fertilizer response		
		nfertilized plots	n	ntransformed	Log scale	n
Aerobic incubation	N0-70	0.638	19	-0.528	-0.630	17
		0.0033		0.0293	0.0067	
	N7-70	0.653	19	-0.529	-0.656	17
		0.0025		0.0289	0.0042	
	N_0;0-70	0.045	15	-0.239	-0.230	13
		0.8732		0.4326	0.4500	
	N_0;7-70	0.298	11	-0.475	-0.479	9
		0.3732		0.1965	0.1922	
PSNT		0.664	19	-0.368	-0.479	17
		0.0019		0.1466	0.0519	
Direct diffusion amino sugar		0.693	19	-0.697	-0.779	17
		0.0010		0.0019	0.0002	
Direct diffusion amino sugar & NH_4^+		0.679	19	-0.702	-0.764	17
		0.0014		0.0017	0.0004	
No-trap method		0.677	19	-0.706	-0.720	17
		0.0014		0.0015	0.0011	
Hot KCl		0.588	19	-0.448	-0.490	17
		0.0082		0.0716	0.0457	
Anaerobic incubation		0.251	19	-0.343	-0.308	17
		0.2994		0.1773	0.2287	
CO_2 flush (3 days)		0.368	19	-0.474	-0.491	17
		0.1206		0.0546	0.0452	
Total C		0.678	19	-0.600	-0.639	17
		0.0014		0.0109	0.0057	
Total N		0.698	19	-0.636	-0.748	17

The other tests were significantly but only moderately well correlated with N uptake, except for the anaerobic incubation and the CO_2 flush, which had no significant correlation with N uptake. The amino sugar method was among a group of tests with a similar performance (Figure 11).

The correlation between N uptake and the different N mineralization indices could be improved when the initial mineral N was added to the N mineralized. This procedure makes sense, as both the mineral N present in spring and the N mineralized during the cropping season contribute to plant nutrition. With this adjustment, the best tests, the amino sugar and the no-trap method, reached R^2 values of 0.65.

Only half of the N mineralization indices were significantly related to the fertilizer response. As the distribution of the fertilizer response more closely followed a logarithmic scale than a normal scale, transforming the data improved the agreement between the N mineralization indices and the fertilizer response (Figures 12 and 13).

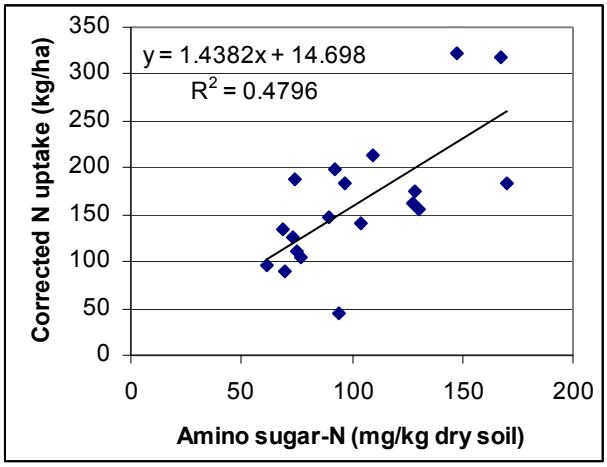


Figure 11. Relationship between soil amino sugar and N uptake in unfertilized plots.

The N mineralization index best related to the transformed fertilizer response was the soil amino sugar content (R^2 = 0.61; Figure 13). Nevertheless, the correlation does not seem to be close enough to be used for accurate fertilization recommendations, although the data look more encouraging when the graph is taken into account. Two fields (Escalon II and III) prevented the relationship from being much closer. While in one field the amino sugar content was too high for the measured fertilizer response, it was too low in the other field. Unfortunately, these two fields were located on the same farm and have a similar crop history.

Adding the mineral nitrogen to the result of the soil tests increased the correlations only slightly. The total soil N was as good a predictor for the crop performance as the better N mineralization indices.

Fig.12.

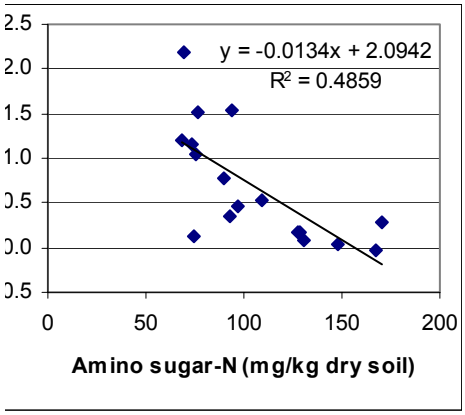
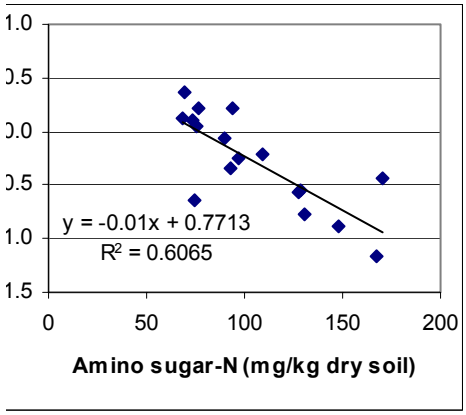


Fig.13.



Figures 12 and 13. Relationship between soil amino sugar and response of corn to N fertilization.

Conclusions

All the soil tests were positively correlated with the N mineralized during the 10-week aerobic incubation. In contrast to conclusions from the greenhouse study using soils from the LTRAS site, in the field study of farm locations throughout the Central Valley, the anaerobic incubation was not superior to the chemical tests. The correlation coefficients for most of the methods were within a relatively small range. Even though the proportion of the total N mineralized varied from 2.1% to 9.5%, the total N was as well correlated with N mineralization as the other soil tests.
With the exception of the aerobic incubation, the N mineralization indices evaluated in this study can be carried out relatively easily and do not require much time, which makes them suitable for use in soil test labs. However, the correlation of soil tests with field results was not close enough to be used for fertilization recommendations. Total soil N content, normally included in standard soil analyses, performed as good as the best N mineralization indices, which agrees with the conclusions of Fox and Piekielek (1978b). Several reasons may be responsible for these moderate correlations:

The soil is only one factor that affects crop performance. While the weather may be less important in California with its hot summers and water supply by irrigation, crop management definitely plays an important role.

The approach chosen to calculate fertilizer response may have resulted in incorrect results in some fields where the corn did not reach its full yield potential. The results would have been more accurate if the plots had received a sufficiently high N application by the researchers.

The number of field sites was very small. One or two fields with different values had a big effect on the overall correlation, as can be seen in the case of the amino sugar test (Figure 7.4). A larger number of field sites would have allowed for multivariate analysis to detect other factors that affect crop performance.

The amino sugar content above which corn doesn't respond to further fertilization was lower in this study than that reported for Illinois by Khan et al. (2001). This may be due to the higher summer temperatures in California, which increase the decomposition rate of organic material and therefore the amount of N mineralized.

This study suggests that a single soil test, taken in spring, is not enough to make accurate N fertilization recommendations. Other factors, such as climate and management must be taken into consideration when these methods are used to aid prediction of N fertilizer requirements (Gianello and Bremner, 1988). Even though dynamic models can integrate all the important factors and should therefore be more successful in predicting the availability of N at a specific site, biological and chemical soil tests will be needed to calibrate models site-independently.

Outreach Activities

The following tasks are addressed in this section.

Task 10, year 2. Facilitate transfer of amino sugar method to commercial soil test lab for comparison of results to UC Davis and to assess issues about industry adoption.

Task 12, year 3. Develop and conduct an outreach program.

Industry presentations

In addition to summaries published in the FREP conference proceedings, we made two workshop presentations that contributed to licensed Pest Control Adviser and Certified Crop Adviser continuing education. These were at workshops sponsored by UC Cooperative Extension Stanislaus Co. (Modesto, Nov. 28, 2005, 30 attending) and the Sutter Yuba chapter of the California Association of Pest Control Advisers (Yuba City, Dec. 5, 2006, 50 attending). Powerpoint presentations in pdf format are available from the project leaders.

Commercial laboratory collaboration

In 2006, we collaborated with two large commercial laboratories ("Lab A" and "Lab D") in California to review interlaboratory variability and the practicality of our modified alkaline hydrolysis soil test procedure for determining "amino sugar N" in a production laboratory environment. 100-gram subsamples of ten soils from our project, air-dried and ground to pass 2 mm screen, were sent by US mail to the two laboratories. Nine of the ten soils were from fields in irrigated annual crop rotations. The other soil was from a field in grass pasture which had been farmed until one year earlier in an annual cropping rotation. One of the samples was from an organically farmed plot on the UC Davis Agronomy Farm. The range of soil pH values is narrower than that found in the region's agricultural soils, but the ranges of soil C contents and textures are typical of Sacramento Valley and northern San Joaquin Valley irrigated agricultural soils (Table 10).

Table 10. Selected properties of soils used in interlaboratory comparison

Soil	pH	% C	Textural class (0-20 cm)
1	6.95	1.83	Loam
2	6.66	1.51	Silt loam
3	8.03	1.13	Silty clay
4	6.89	2.10	Loam
5	6.95	1.49	Clay loam
6	6.98	0.53	Loamy sand
7	7.28	1.77	Silty clay
8	7.31	1.05	Clay loam
9	7.22	1.24	Loam
10	6.94	0.72	Clay loam

The two laboratories agreed to conduct the analysis on duplicate subsamples using the analytical protocol that we provided to them. The values obtained in our laboratory were not revealed to the collaborators until after they had completed their analyses.

Laboratory D initially reported values approximately one-third lower than both Laboratory A and our laboratory (data not shown). This was suspected to be due to use of insufficient buffer reagent to neutralize all the acid during final ammonia analysis (with the laboratory's Lachat procedure). This was confirmed by pH values measured in the hydrolysate. Additional buffer reagent was added during a second run, resulting in the correct values. The two commercial laboratories obtained results that were very close to those in our laboratory (Fig. 14). For the two soil samples with the highest values, our laboratory obtained values 17% and 8% below the mean obtained by the commercial laboratories. Coefficients of variation for duplicate analyses averaged 3.9% for Lab A and 4.5% for Lab D, well within industry standards. Both laboratories reported that the procedure was easy to run and could be used within the constraints of their normal operations.

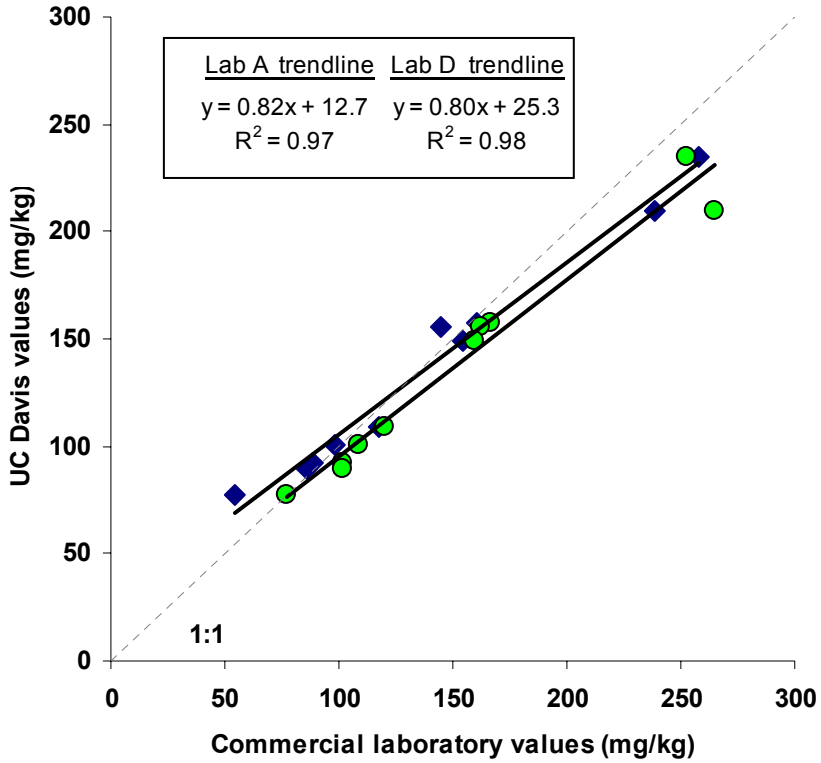


Fig. 14. Comparison of values obtained by two commercial laboratories and UC Davis using the modified alkaline hydrolysis procedure developed by this project for "amino sugar N".

Presentations (July – December 2006)

Pettygrove, S. and W.R. Horwath. Practical Soil Test methods for Predicting Nitrogen Mineraliztion. 2006. UC Cooperative Extention/CAPCA Soil Ferttility Meeting. Yuba City, CA Dec. 5, 2006.

Practical Soil Test Methods for Predicting N Mineralization. 2006. California Dept. Food and Agriculture Fertilizer Research and Education Program. 14th Annual Fertilizer Research and Education Program Conference. November 29, 2006, Monterey, CA.

Managing Soil Ecosystems. 2006. . Sustainable Ag Expo 2006, Monterrey, CA.

Publications

Geisseler, D., S. Pettygrove and W.R. Horwath. 2007. Modification of the Amino Sugar Test to Predict Soil Nitrogen Mineraliztion. Soil Science Society of America Journal. Submitted.

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